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A LOCUS WITH A MATERNAL EFFECT ON EMBRYONIC DEVELOPMENT IN *DROSOPHILA MELANOGASTER*

by

PATRICIA ANN ROMANS

A THESIS

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FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled A Locus with a Maternal Effect on Embryonic Development in *Drosophila melanogaster*, submitted by Patricia Ann Romans in partial fulfilment of the requirements for the degree of Master of Science.

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Date....September.10.,.1973.

ABSTRACT

A new locus, miel(1)R1, in Drosophila melanogaster which has a maternal effect on embryonic development has been located in a screen for female sterile mutants. This locus is X-linked. It maps between 0.21 and 0.83, and has been assigned to the cytological region between salivary gland chromosome bands 2D1 and 3A1. Two mutant alleles have been identified and studied. One of these alleles, miel(1)R1^{r}, is genetically rescuable: when homozygous mutant females are mated to mutant males all the embryos die, but when such females are mated to wild-type males, some heterozygous female offspring are produced. It is thought that a defect in the egg cytoplasm resulting from the homozygous mutant condition of the mother is compensated for by the action of a wild-type allele contributed by the sperm. The other allele, miel(1)R1^{o}, which also causes maternally influenced embryonic lethality, is not genetically rescuable. Genetic rescue is dominant at this locus.

The characteristics of the miel(1)R1 locus as revealed by the studies on the two mutant alleles have been compared with other loci which have genetically rescuable alleles. The results on all these loci are consistent with the hypothesis that these mutants are defective in macromolecules involved in the general metabolism of the fly rather than in the structural elements of morphogenesis. However, these gene products are essential to support morphogenesis.

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Maye Mullins mapped many of the female sterile mutations during the summer of 1972.

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INTRODUCTION

One of the major problems of embryology is determining the maternal contribution to the developing embryo and the details of the switch-over to selective activation of the embryonic genome.

This problem has been approached experimentally in sea urchins and in various amphibian species for many years, beginning with the species hybrid experiments of Boveri in 1889. (For reviews of experimental embryology, see Davidson, 1968; Balinsky, 1970). The first species hybrid experiments established that embryonic development is controlled by nuclear factors. Later experiments suggested most of our present concepts of embryogenesis: the idea of maternal templates functioning early in development, and the concept that the onset of differentiation is related to the time of activation of the embryonic genome (Davidson, 1968).

More recent biochemical studies have refined these observations. There is direct evidence that early embryogenesis relies on maternal RNAs deposited in the egg during oogenesis. Embryonic genes begin to be activated during blastulation in amphibian embryos. This is probably in response to the cytoplasmic environment in which individual nuclei find themselves. RNA synthesized at this time is essential for gastrulation, although maternal information is not expressed until much later.

These studies on embryogenesis have been extended by the identification of mutations causing abnormal embryogenesis in various species. In view of the sophisticated genetic analysis to which Drosophila melanogaster can be subjected, it is not surprising that most progress has been made on this organism. The embryogenesis of wild-type Drosophila has been exhaustively described (Poulson, 1950; Sonnenblick, 1950), and the main events have been summarized in Table 1. These are given mainly as a basis of reference for further discussions. More recently attempts have been made to describe Drosophila embryogenesis causally on the basis of experimental manipulations and mutant studies (notably Wright, 1970; Counce, 1973).

Two kinds of mutations are known to affect embryogenesis in Drosophila. These are the recessive embryonic lethal mutations and certain female sterile mutations. Female sterile mutants constitute one of the most promising classes of mutants with abnormal embryogenesis, although it is now clear that many female sterile mutants do not have defective embryogenesis (Gill, 1963; Bakken, 1973). In those female steriles where embryogenesis is defective, this is now known to be the result of a maternal defect in oogenesis.

I. THE MATERNAL CONTRIBUTION TO EMBRYONIC DEVELOPMENT

Cytological studies on a variety of recessive embryonic lethal mutants (Wright, 1970) show that death seldom occurs before

Table 1. Timetable of 'landmark' events in the development of Drosophila melanogaster embryos.[1]

Time (in hours)	Stages at 23-25° C
¼	End of telophase 2, conjugation of male and female pronuclei
1/3 - 1½	Synchronous cleavage mitoses (8 divisions)
1½ - 2	Migration of cleavage nuclei; pole cell formation
2 - 2½	Syncytial blastoderm; 3 synchronous blastoderm mitoses
2 - 2½ - 3	Cellular blastoderm formation, pregastrular contraction of embryo
3 - 3½	Early gastrulation, ventral furrow formation
4 - 5	Germ band extension, anterior & posterior invaginations, dorsal folds
5	Extended germ band, primitive gut, large neuroblasts
6	Mesoderm segmented, stomodaeum & protodaeum deep
7	Tracheal invaginations
8	Segmentation of head and trunk, muscle attachment
9 - 10	Shortening of embryo
10 - 12	Involution of head, dorsal closure
13	Midgut constrictions
14 - 16	Condensation of ventral nervous system; muscular movements
20	Air in tracheae
22	Hatching

[1] Data From Sonnenblick, 1950, pp 154-5; Poulson, 1950, p 242

blastoderm formation. Usually death occurs toward the time of larval hatch. The experimental study of von Borstel and Rekemeyer (1958) showed that Drosophila embryos lacking sex chromosomes, and thus nucleoli, ceased development just prior to cellurlarization of the blastoderm. This indicates that there is probably no sex chromosome gene function required before blastoderm formation. It also suggests that pre-blastoderm protein synthesis occurs on maternally-derived ribosomes. This is further substantiated by the observation that during normal embryogenesis, nucleoli simultaneously appear just prior to cell membrane formation around the blastoderm nuclei (Sonnenblick, 1950). Von Borstel and Rekemeyer (1959), and Scriba (cited in Wright, 1970) have also tested autosomal aneuploids to determine when embryonic autosomal gene activity becomes necessary. The general conclusion to be inferred from all these studies is that there is no embryonic gene activity required before blastoderm formation.

Direct evidence from studies involving non-mutant embryos supports this conclusion. As has been previously mentioned, nucleoli first appear simultaneously in all nuclei at the egg cortex just prior to any visible cell membrane formation. The switch-over from embryonic histones (thought to be maternally derived) to adult histones has been shown histochemically to occur immediately prior to cellularization and the appearance of nucleoli (Das et al, 1964). These authors have also noted that the chromosomes of cleavage nuclei contain little if any RNA prior to the histone change. These results

imply that some basic change in the regulation of the genetic material occurs at this time. A variety of enzyme studies (Wright and Shaw, 1970; Wright, 1970) has shown that the activity of paternal forms of enzymes appears at various times throughout embryonic development, but in each case, subsequent to gastrulation. The earliest yet known paternal enzyme activity is that of xanthine dehydrogenase, the product of the rosy locus (Sayles, et al, 1973). It is detectable by a sensitive fluorescence assay at gastrulation.

II. THE NATURE OF THE MATERNAL CONTRIBUTION

The accumulation of the macromolecules on which preblastoderm embryogenesis depends occurs during the later stages of oogenesis, namely King's stages 8 through 11 (King, 1970) when chorion deposition takes place (Table 2). In Drosophila, the oocyte appears to be rather passive with respect to synthesis of its own macromolecular components. The diplotene chromosomes of Drosophila are tightly contracted into a karyosome from stages 2 to 3 onwards until egg deposition (Table 2; King, 1970; Counce, 1973) and are thought to be totally heterochromatic (Chandley, 1966). This is in contrast to amphibia whose diplotene chromosomes are in the expanded lampbrush configuration and are believed to be actively synthesizing RNA (Brown and Dawid, 1969); Davidson, 1968).

These macromolecules which accumulate in the developing oocyte are believed to have three possible immediate sources other than the oocyte itself: the nurse cells, the follicle cells, and the haemolymph.

Table 2. Developmental Stages of Oogenesis in *Drosophila melanogaster* [a]

Stage No.	Identifying features	Oocyte	Nurse cells	Follicle cells
1	16-Cell germarial cyst in germarium	Synaptinemal complexes form in two pro-oocyte then degenerate in one	Oocyte:nurse-cell size ratio is 1:1 and will be until stage 8	Form monolayer around germarium
2	Follicle = ellipse; egg chamber enters vitellarium		Nurse cells begin polyploidization	Cuboidal shape; divide to keep up with cyst growth
3	Nurse-cell nuclei, and oocyte's beginning karyosome		Nuclei show thick threads of chromatin	
4	Karyosome is compact, follicle is round, nurse-cell nuclei prominent	Oocyte chromatin condenses to characteristic Feulgen (+) karyosome	Nuclei contain densely stained bulbs of chromatin	
5	Follicle is round, nurse-cell nuclei prominent		½ the nuclei = stage 4, ½ the nuclei = stage 6	
6			All nuclei are homogeneous and densely staining	cease division and begin growth
7	First elongate egg chamber. Last previtellogenic stage	Oocyte: nurse-cell size ratio still 1:1	Nuclei show size gradient	Begin columnarization over oocyte
8	Yolk deposition begins in oocyte	Oocyte is larger than largest nurse cell and will continue to grow	Polyploidy now = 128-256 N	Colmnarization continues over oocyte

Table 2 continued

Stage No.	Identifying features	Oocyte	Nurse cells	Follicle cells
9	Border cells near oocyte		Nurse cells at maximum DNA content (512 N)	Border cell migration to oocyte Cells over nurse cells begin to be squamous
10	Vitelline membrane	Oocyte = ½ of chamber volume	Nuclei are maximum size	Vitelline membrane formation
11	Chorion formation	Oocyte = 3/4 of chamber volume. Karyosome condenses	Nurse cells empty into egg	Columnar cells begin to shorten
12	Shrunken nurse cells form anterior cap	Oocyte is fully grown	Condensed nuclei form anterior cap outside oocyte	Inner endochorion formation
				Micropyle and chorionic filaments begun
13	Micropylar apparatus is completed			Outer endochorion formation
14	Chorionic filaments are complete. Egg is mature	Nuclear membrane disappears, leaving condensed karyosphere. Meiosis resumed after leaving ovary		Exochorion formation

[a]This table has been modified from a table in Bakken, 1973

Female sterile mutations whose primary effect appears to be in nurse cells and in follicle cells do exist and have been studied, mainly by King and his coworkers (see King, 1970 for review). The heamolymph would seem to be primarily a transport tissue and it probably conveys nutritive substances to the developing oocyte for storage and utilization during embryogenesis. Female specific yolk proteins isolated from the haemolymph of certain moths (for example, Pan and Wyatt, 1971) and of mosquitoes (Roth and Porter, 1964) can also be detected in ovaries which are in the vitellogenic stages. Circumstantial evidence for the role of haemolymph-born proteins in the vitellogenesis of Drosophila is the non-autonomous behaviour of transplanted ovaries of the mutant apterous^{4} which has yolk-deficient oocytes (see King, 1970).

Biochemical studies carried out over the last fifteen years give an indication of the nature and variety of the substances transported into the maturing oocyte. These are: 1) nucleic acid precursors, free bases, and nucleosides. Nucleotides have not been found (levenbook et al., 1958) 2) ribosomes and RNA. The autoradiography experiments of Sirlin and Jacob (1960) provide evidence for the manufacture of these substances in nurse cells and their subsequent transport to the oocyte. The transfer of mRNA to the Drosophila oocyte in the same fashion has not been directly demonstrated. 3) the cleavage type histone. This histone is a lysine-rich egg-specific histone which has different staining properties from the adult histone that appears just before blastoderm cellularization (Das et al., 1964).

4) other basic proteins. These basic proteins are found associated with RNA and are thought to be the structural proteins of ribosomes. They are probably exported from nurse cells into the oocyte (Davenport and Davenport, 1967). 5) precursors of the oocyte membranes, the vitelline membrane and the chorion. These appear to originate as secretions of the follicle cells positioned over the oocyte (King, 1970). 6) certain enzymes which have high activity early in embryogenesis. These include 6-phosphogluconate dehydrogenase (Wright and Shaw, 1970), aldehyde oxidase (Dickinson, 1971), Esterase-6 (Wright, cited in Wright, 1970), and the product of the maroon-like locus, which may be a coenzymme of xanthine dehydrogenase (Glassman and Mitchell, cited in Sayles et al., 1973). 7) yolk spheres. These spheres appear to form in the oocyte itself and are of two main types. α-yolk spheres are membrane bound and contain mainly protein and lipid. Undoubtedly some of the yolk protein originates in the haemolymph. However part of the protein appears to be synthesized in the oocyte on endoplasmic reticulum to which these vesicles are attached. The origin of the endoplasmic reticulum and its attached ribosomes is the nurse cells (King and Devine, 1959; King, 1970). β - yolk spheres are not membrane bound and appear in the oocyte at stage 13 (Table 2) after chorion deposition. They contain various carbohydrates and saturated lipids (King, 1970). 8) oocyte mitochondria. These originate in nurse cells, but it has been shown that mitochondrial DNA replicates in the oocyte itself (see King, 1970).

III MUTATIONS WITH A MATERNAL EFFECT ON EMBRYOGENESIS

Mutations affecting the production of any of the substances listed above or the processes by which these substances enter the oocyte will result in female sterility. The fertilized eggs of such mutants will die during embryogenesis, and I propose that such mutants be called "miel", an acronym for maternally influenced embryonic lethal.

The accumulated data on female sterile mutants in Drosophila support the generalization made earlier that the genome of the embryo is relatively inactive prior to blastoderm formation. Studies on female sterile mutants (King, 1970; Wright, 1970; Kaplan et al., 1970; and Bakken, 1973) indicate that in the majority of cases where the sterility is actually due to embryonic death, development ceases before blastoderm formation. Normal early embryonic development depends almost exclusively on substances contributed to the oocyte during oogenesis. From gastrulation onwards the embryo relies increasingly on its own genetic material.

In view of the major maternal contribution to pre-blastoderm development, a special interest attaches to those few miel mutants whose wild-type product is required later in embryogenesis. Some of these mutations have the property that they are a kind of conditional lethal. In this case, the conditionality depends on the genetic constitution of the embryo. There exist at least three classes of these conditional miel mutants which have not previously been distinguished from each other.

In the first case, the amount and nature of the sex-chromosome heterochromatin influence the lethality of an embryo. Two mutants of this type are known: abnormal oocyte (abo) (Sandler, 1970; 1972) and daughterless (da) (Sandler, 1972). Both are second chromosome recessive mutations which act in females to give almost unisexual progenies. The effects of both mutants can be ameliorated by increasing the amount of sex-chromosome heterochromatin contributed to the embryo by either the mother or father or both. Mange and Sandler (1973) report that an enhancer of da, E(da) acts as a suppressor of the abo effect, and consider that the two mutations may be functionally related.

The mutant sonless (snl) an X-chromosome recessive mutation described by Colaianne and Bell (1970; 1972) represents a second type of conditional miel mutation. Homozygous snl/snl females produce almost no progeny when mated to snl/Y males, and no sons and fewer daughters than expected when mated to +/Y males. The death of the embryo of snl females is conditional upon maleness and the genotype (snl/+ or snl/snl) of the embryo: more daughters survive when the embryonic genotype is snl/+. Patroclinous (+/0) males survive, as do genotypically snl/Y sons which are homozygous for the feminizing mutant doublesex. But, low numbers of snl/+ or snl/snl daughters which are homozygous for the mutant transformer that causes genotypic females to have a sterile male phenotype (Colaianne and Bell, 1972), survive. It will be seen that the conditionality exhibited by snl may be a special case of the type of conditional miel mutant described below, due to the nature of the snl locus itself. The relatively poorer survival of female progeny in the cross snl/snl x snl/Y than in the cross snl/snl x +/Y suggests this possibility.

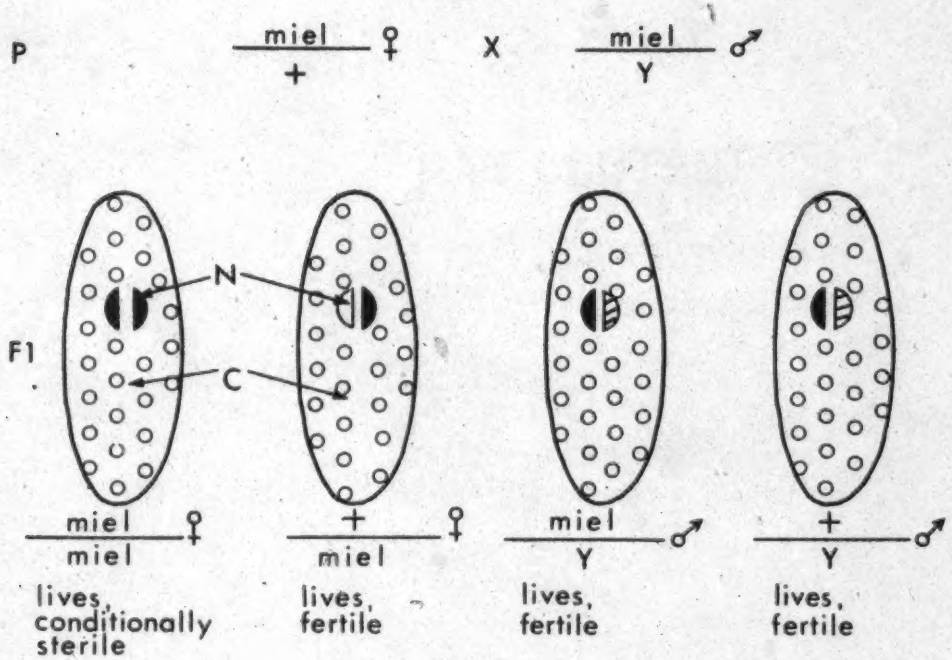
A third type of maternally influenced conditional embryonic lethality occurs when embryonic survival is dependent upon the contribution to the zygote of the wild-type allele of the locus in question. I have called this condition "genetic rescue". The pattern of lethality of genetically rescuable miel mutations is shown in Figure 1. This class of mutants is now represented by a number of different loci, all of which are X-linked. Kaplan et al. (1970) recovered nine such mutations, l(1)mat 1, etc., at a fequency of 0.73% of X-linked recessive lethals induced by EMS. Six of these mutations were found to represent six different loci, but these mutants have not been studied in detail. Three more such mutants, all exhibiting temperature sensitive lethality, have been reported recently (Mayoh and Suzuki, 1973; Tarasoff and Suzuki, 1970; and Wright, 1973). The female sterility of these mutants has not been quantified. Six genetically rescuable miel mutants have been well characterized. These six were all isolated by morphological characteristics except one (yea 1-1) which was identified first as a nutritional conditional mutant (Falk, 1973). The genetically rescuable miel phenotype of these mutants was discovered subsequently. These mutants are cinammon, (cin); deep orange, (dor); almondex, (amx); yeast 1-1, (yea 1-1); rudimentary, (r); and fused, (fu). These mutants will be described briefly with attention drawn to the similarities in phenotype they exhibit.

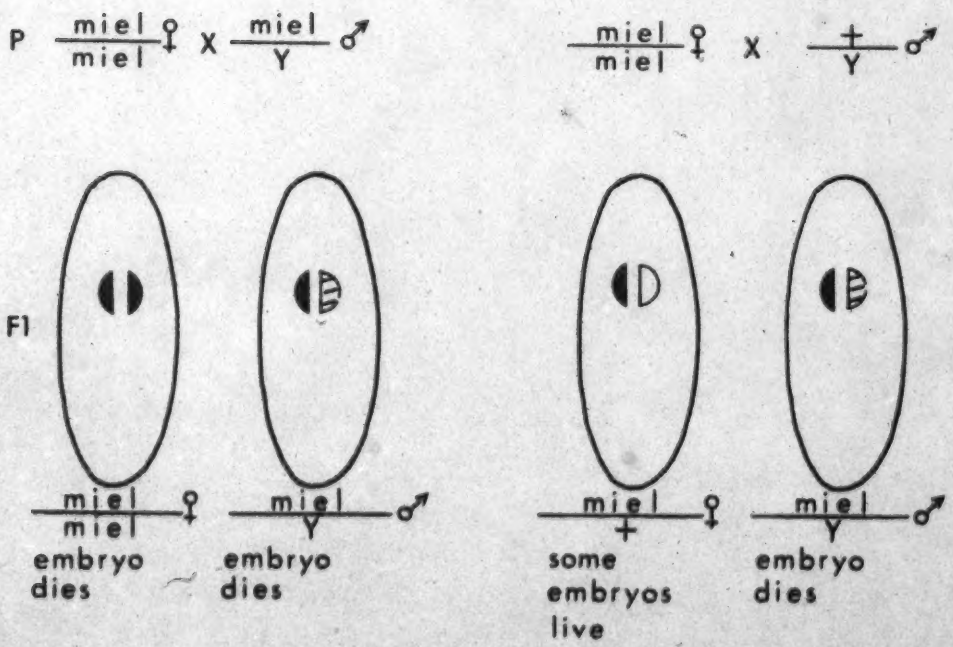
A. fused.

fu (1-59.5) is characterized by abnormal wing veination, ovarian tumours, and female sterility. The genetically rescuable maternally influenced embryonic lethal phenotype of fu has been described in detail by Lynch (1919), Counce (1956b), Clancy and Beadle (see King, 1970), and by Fausto-Sterling (1971a,b). Lynch

Figure 1. The sterility pattern of mutant females which produce maternally influenced embryonic lethal embryos: genetic rescue, a conditional phenomenon.

Black nuclei contain the miel allele, white nuclei contain the + allele. The Y chromosome is indicated by hatching. The egg cytoplasm of progeny of heterozygous flies is shaded with circles to show that it contains the maternally derived miel+ product and is capable of normal development. Unshaded cytoplasm of eggs produced by mutant females indicate that it is deficient in the maternally derived miel+ factor. C, cytoplasm; N, nucleus.


P
miel ♀ / +
X
miel ♂ / Y
F1
N
C
miel / miel ♀
+ / miel ♀
miel / Y ♂
+ / Y ♂
lives, conditionally sterile
lives, fertile
lives, fertile
lives, fertile


P
miel / miel ♀ X miel / Y ♂
miel / miel ♀ X + / Y ♂
F1
miel / miel ♀
miel / Y ♂
miel / + ♀
miel / Y ♂
embryo dies
embryo dies
some embryos live
embryo dies

discovered and correctly described the genetic rescue in her study of this mutant. Lethal embryos develop until late in embryogenesis. Segmentation of the embryo takes place, but is more abnormal ventrally than dorsally (Counce, 1956b). In the three fu alleles she studied, the damage to embryos and overall fertility differed in degree. Rescued fu/+ adult females have a high incidence of abnormalities of abdominal segmentation.

Fausto-Sterling (1971b) showed for one fu allele that it is probably that the fertilization of a fu egg by a +-bearing sperm is not always sufficient to ensure survival. A maximum of 70% of the expected heterozygous female embryos hatched. She made the suggestion that the mechanism which controls the activation of the paternal fu+ allele must be imprecise; that is, sometimes it is either activated too late, or it is not activated at all.

The sterility of fu ovaries is autonomous (Clancy and Beadle, cited in King, 1970: as are others of the pleiotropic effects of the mutant. Fausto-Sterling (1971a), in a study of fu gynandromorphs, has provided evidence for the cell-autonomy of the fu defect, and hence post-blastoderm gene action.

B. rudimentary.

The wing phenotype and female sterility of r (1-54.5) were originally described by Morgan (1912, 1915) and the nature of the miel effect correctly interpreted by Lynch (1919). As with fu, lethal r embryos die late in embryogenesis, apparently at the time of tissue differentiation (13 - 16 hours; Table 1; Counce, 1956c).

Both the wing phenotype (Morgan, 1912; Green, 1963) and the miel effect (Fausto-Sterling, 1971a) of r are cell autonomous.

Recently Norby(1970) showed that r mutants are defective in pyrimidine biosynthesis. Subsequently, (1973) he showed that of two mutant alleles which complement one another (Carlson, 1971), one has almost no aspartate transcarbamylase activity, while the other has almost wild-type activity. A third r allele which complements with neither of the previous two shows low enzyme activity.

Many r alleles are known whose effects differ in severity: some of the r alleles studied by Lynch allow the production of small numbers of male and female offspring from homozygote crosses. Falk (1973) has isolated several pryimidine auxotrophs which map at r. Many of these show the wing and sterility phenotypes, but others are recognizable as r alleles only by their pyrimidine requirement, supplementability by carbamyl aspartate, and the slight wing effect exhibited in flies which are heterozygous for a totally non-complementing r allele (Carlson, 1971). It is possible to establish a rank ordering of the mutant alleles based on the severity of any one of the pleiotropic phenotypic effects.

The absence of tissue specific developmental abnormalities found in non-rescued r embryos is not unexpected if a process fundamental to survival such as pyrimidine biosynthesis has been disturbed (Norby, 1970). In this context, Bahn,(1970) has reported that fertility of r females increases when they are fed a pyrimidine enriched medium.

C. deep orange.

The deep orange locus (1-0.3) has a variety of mutant alleles

which exhibit phenotypes ranging from recessive larval lethality through female sterility, to a slight effect on eye colour (Bischoff and Lucchesi, 1971). Like r, dor alleles correlate the severity of expression of their various mutant phenotypes.

Counce (1956a, 1969) and Hildreth and Lucchesi (1967) have investigated the development of non-rescued dor embryos. Most fertilized embryos develop past early gastrulation and may show features of very late embryos, although blastoderm cellularization may be abnormal. The fertility of dor females and males and the damage to embryos appears to be extremely sensitive to variation in environment and genetic background.

Both the eye colour and rescuable miel effect are autonomous in their respective adult structures, although evidence for the latter is scanty (Garen and Gehring, 1972). These authors experimentally extended development in lethal dor embryos by injecting them with cytoplasm of wild-type unfertilized eggs within about three-quarters of an hour after deposition.

The precise biochemical function of the dor locus is not known. However, dor flies have altered pteridine pigments. Pteridines function as cofactors in hydroxylation reactions, in folic acid acid biosynthesis, and also in pigment formation. Their function in intermediary metabolism is an important one.

D. almondex.

almondex (1-27.7) is a miel mutant in which only slight rescue from the cytoplasmic damage to the egg is effected by the + allele contributed by the sperm. The bulk of the analysis of the sterility phenotype is the work of Shannon (1972a;b; 1973). amx differs from other mutants of this class in several respects. 1) Homozygous females and hemizygous males exhibit no pleiotropy of mutant effects.

2) Only one mutant allele is known. 3) The genetic rescue is very slight: about 5% of expected rescued heterozygous female progeny eclosed at 27°C (Shannon, 1972b). 4) amx appears to be a cold-sensitive mutant: the fertility of amx at 27°C is about 100 times that of females kept at 23°C. 5) Fertility increases with aging of the female fly. 6) The mutant phenotype is non-leaky. No progeny escape lethality in the cross amx/amx x amx/Y.

Like fu, rescued females show very high rates (about 80%) of abnormalities involving the thorax and/or sternite segmentation (Shannon, 1972b).

E. cinammon.

cinammon (1-0.0) described by Baker (1973) is a non-temperature sensitive miel mutant. Some cin progeny do escape the lethal effect. In contrast to most other rescuable miel mutants, notably amx, the genetic rescue of cin embryos by a + paternal allele is virtually complete, and the heterozygous female offspring are normally fertile. Baker has shown that progeny rescue is independent of sex in that a y^+ cin^+.Y contributed by the father rescues male embryos. Of the described mutants, this experiment has been performed only for cin. However, this result will probably obtain for all the other miel mutants of this class, except sonless.

This mutant allele also produces a maternal effect on eye pigmentation. Escaper progeny have a red-brown eye colour. cin flies lack detectable xanthine dehydrogenase activity and accumulate its pteridine and purine substrates. It has been shown that the embryonic lethality is not a consequence of the accumulated pigments by the result that homozygous cin females that were also phenotypically white ie. w/w or cn/cn; bw/bw exhibited the maternal effect.

The wild-type product of cin is probably not a sub-unit

of xanthine dehydrogenase since rosy is known to be the structural gene for the enzyme, and homozygous ry flies are fertile. cin^{+} could, however, specify a second activity of the molecule, but a more likely explanation is that, like the maroon-like^{+} substance which is also maternally inherited (but non-lethal in the mutant), it may be a cofactor.

The lethality of cin progeny of cin mothers has several possible biochemical explanations. Some of these are similar to those proposed for dor because of the accumulation of pteridines.

F. yeast 1-1.

yea 1-1 is an auxotrophic mutation which maps at 1-36.6 (Falk, 1973). This mutant shows the same pattern of female sterility as that described for the previous mutants, and is also a cold-sensitive recessive lethal. Mohan and Suzuki (1973) have reported a cold-sensitive recessive lethal mutant, l(1)MH23^{cs}, having the genetically rescuable miel phenotype. It maps very close to yea 1-1 and may possibly be an allele.

IV THE miel(1)R1 LOCUS

In the last few years several Drosophila workers have set out to isolate large numbers of female sterile mutants (Gill, 1963; Bakken, 1973; Mohler, 1973). This thesis reports a new screen for such mutants. Twenty-nine X-linked recessive female sterile and semi-sterile mutations were isolated. The preliminary characterization of these mutants is given in the Appendix to this thesis.

Two of these mutations were found to be alleles of a new locus which has a maternal effect on embryonic development. One of

the alleles is a genetically rescuable maternally influenced embryonic lethal. This mutant, stock number L207, was named mie1(1)R1^{r}. The designation "mie1" has already been described. The rest of the designation follows convention for the naming of lethals and the super-script r refers to the fact that this allele is genetically rescuable. The other allele was the only other mie1 mutation isolated where embryos produced by homozygous mutant females die subsequent to gastrulation. This stock, number M163, was given the mutant name mie1(1)R1^{o} on the basis of allelism: o signifies obligate lethality of the affected embryos.

This study presents genetic results which delineate the phenotypes of mie1(1)R1^{r} and mie1(1)R1^{o} mutant flies. This locus is compared to the six other mie1 loci described above which have genetically rescuable alleles.

The results raise the possibility that activation of paternal genes may be dependent on the cytoplasmic environment. The system can be extended to distinguish experimentally the specific contributions of the paternal and maternal chromosomes to embryogenesis.

MATERIALS AND METHODS

I. *DROSOPHILA* STOCKS

The following list describes the *Drosophila* stocks employed in these studies. Details of the mutant phenotypes may be found in Lindsley and Grell (1968). The duplication and deficiency stocks are described in greater detail in Table 7 and Figure 5.

Stock	Description	Source
+ (Amherst)	Amherst wild-type, a highly inbred line. (see Drosophila Information Service, 1968, stock 1.)	Amherst College
Basc	In(1)sc^{SIL}sc^{8R+S},sc^{SI}sc^{8}w^{a}B w - apricot eyes sc - scute bristles B - Bar eyes	Amherst College
FM7b	Ins(1)sc^{8}+15D-E; 20A-B+dl 49, y^{31d}sc^{8}w^{a}1z^{sp}B y - yellow body 1z - lozenge eyes, female sterile (Merriam, 1969).	California Institute of Technology
+ (Oregon-R)	Oregon-R wild-type	California Institute of Technology
y cv v f	cv - crossveinless wings v - vermilion eyes f - forked bristles	California Institute of Technology
y w	w - white eyes	
w sn^{3}	sn^{3} - singed bristles, female Fertile allele	University of Alberta

dor/Basc	dor - deep orange eyes, genetically rescuable maternally influenced embryonic lethal	University of Washington
dor^1/FM6	dor^1 - larval lethal allele of dor	California Institute of Technology
yea 3-1	yea 3-1 - a yeast requiring auxotrophic mutant, female semi-sterile (see Falk, 1973).	University of Alberta
Pgd^-	Pgd^- - 6-phosphogluconate dehydrogenase activity deficient	California Institute of Technology
$\overline{XY}$, w v f B	$\overline{XY}$ - $Y^{S}X \cdot Y^{L}$, w v f B	University of Alberta
y sc gt^{X11}/ $w^+.Y$/ yf:=	gt^{X11} - giant larva, larval lethal (see Kaufman, 1973). $w^+.Y$ - a Y chromosome with the white region of the X inserted into Y^L. yf:= - C(1)RM, y f, two X chromosomes attached together, marked by y and f.	T.C. Kaufman
Df(1)X12,y sc/ $w^+.Y$/yf:=	Df(1)X12 - Deficiency (1) deficient for most of the region from z to w. (see Judd et al, 1972).	T.C. Kaufman
$Df(1)w^{rJ1}$,y^2sn^3/ $w^+.Y$/ yf:=	Df(1) w^{rJ1} - Deficiency (1) deficient for the region from z to w. (see Judd et al, 1972).	T.C. Kaufman

The FM7b stock was backcrossed several times into the Amherst line before it was used to balance a mutant. Stocks succeeding FM7b on the list were used only for one or two experiments; no effort was made to make them isogenic with the

Amherst line.

II. MEDIA AND REARING CONDITIONS

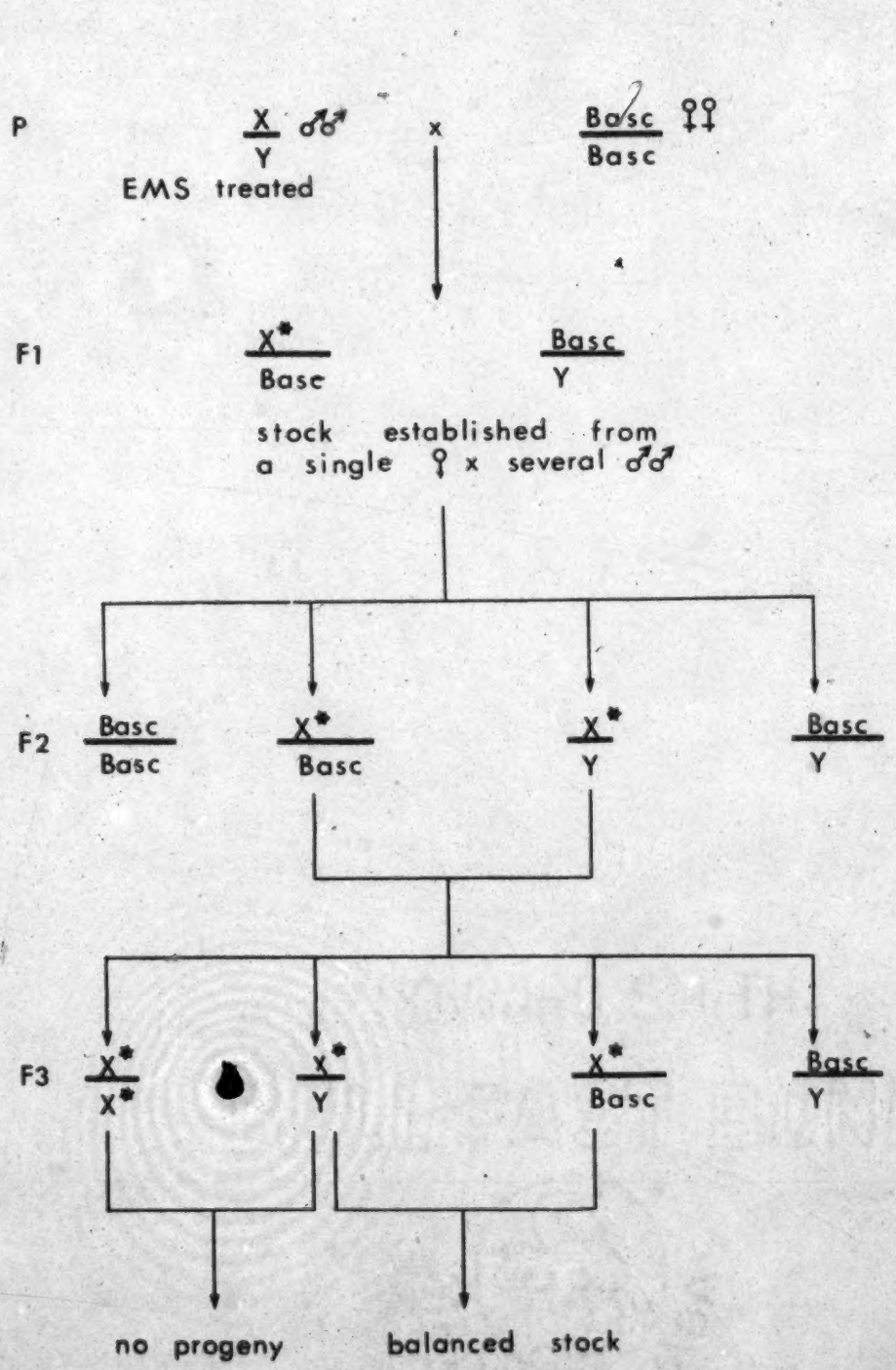
Stocks of flies were reared on the yeast-agar medium of Nash and Bell (1968) in quarter-pint milk bottles at 25°C ±½°C. For experimental crosses, flies were reared in shell vials, usually one female crossed with two or three males. Where eggs were to be counted, flies were placed in vials of medium to which grape-juice concentrate had been added before pouring (see von Borstel and Fine, 1963). Flies used in cytological studies were fed for four or five days prior to the experiment on a paste of live yeast smeared inside bottles of regular medium.

III. PRODUCTION OF MUTANTS

Since it is known that the expression of female sterility in some mutants is very sensitive to differences in genetic background, as well as to environmental changes (for example, see Counce, 1969), mutations were induced in Amherst wild-type flies. These flies probably are nearly isogenic.

Mutations were induced by feeding 24-48 hour old adult male flies on 6.4 mM ethyl methanesulfonate according to the method of Lewis and Bacher (1968). The treated X-chromosomes were then made homozygous using the Muller-5 (Basc) technique (Spencer and Stern, 1948; Figure 2). All the mutants were balanced against Basc or FM7b, in stocks containing autosomes derived

Figure 2. Protocol for production and Isolation of X-linked female sterile mutations: the Muller-5 technique.


P
X
Y
♂♂
EMS treated
x
Basc
Basc
♀♀
F1
X*
Base
Basc
Y
stock established from
a single ♀ x several ♂♂
F2
Basc
Basc
X*
Basc
X*
Y
Basc
Y
F3
X*
X*
X*
Y
X*
Basc
Basc
Y
no progeny
balanced stock

previously from the Amherst line.

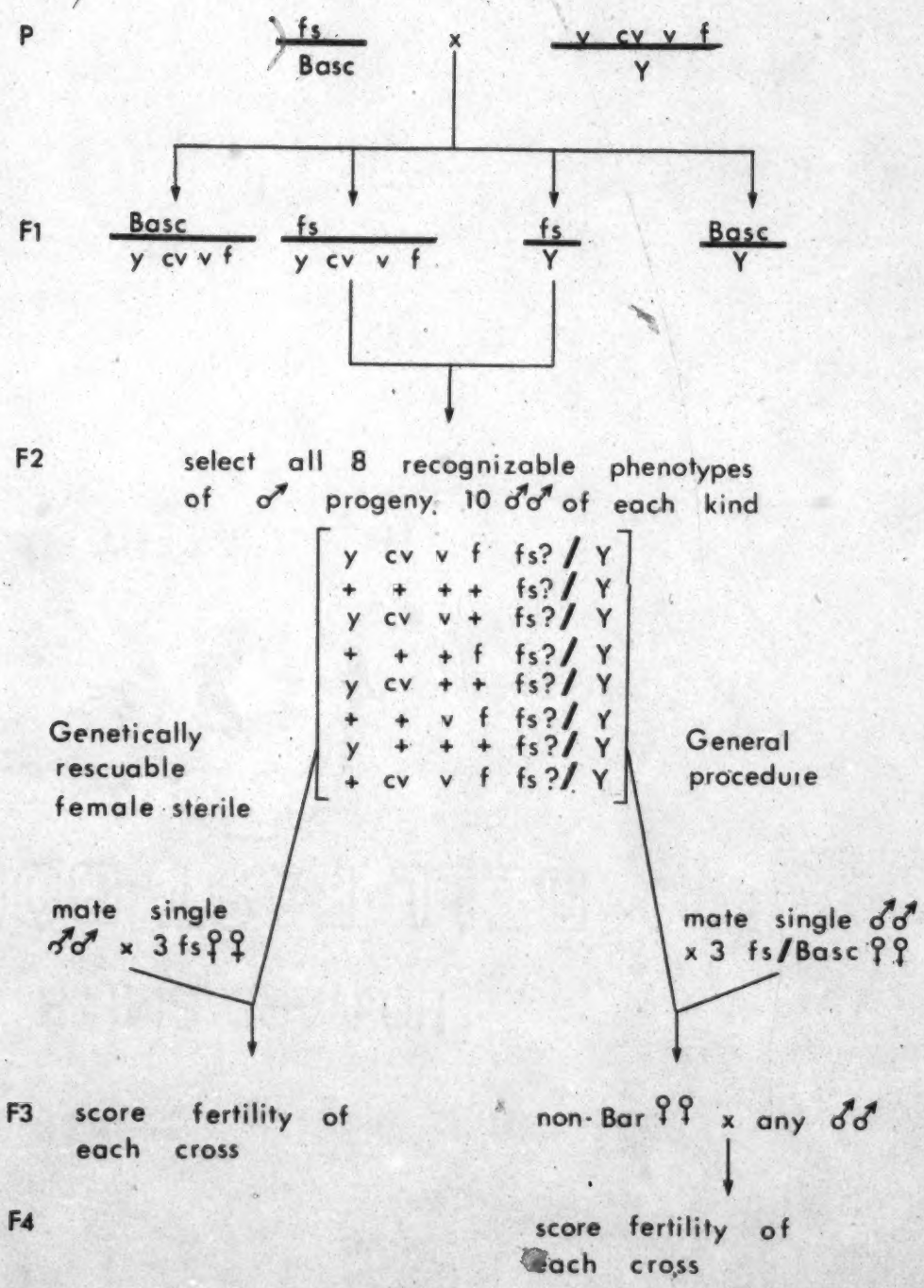
IV. MAPPING

A. Recombination Mapping

Initially the two mutations, mie1(1)R1^{r} and mie1(1)R1^{o} were mapped to a region by testing with the multiply-marked X-chromosome y cv v f: y 1-0.0, cv 1-13.7, v 1-33.0, f 1-56.7. The breeding scheme for mapping X-linked female sterile (fs) mutations is shown in Figure 3, and includes protocols for both the non-rescuable and rescuable types. The general procedure which must be followed for non-rescuable mutations involves carrying the protocol for mapping any X-linked recessive gene an extra generation and scoring the non-Bar females of the F3 for sterility (homozygosity for the female sterile mutation) or fertility (heterozygosity for the female sterile mutation). The procedure may be shortened one generation in the case of a rescuable female sterile mutation: F2 males are singly mated to females homozygous for the mutation and the fertility of each cross is scored.

Subsequently it was desired to obtain a more accurate location of the mie1(1)R1 locus, and mie1(1)R1^{r} was remapped using the y w marker chromosome: y 1-0.0, w 1-1.5, since the locus had been located to this region in the previous test. In this experiment the shortened procedure was used (see Figure 3 for analogy).

Figure 3. Procedure for mapping an X-linked female sterile mutant.


P
fs / Basc
x
y cv v f / Y
F1
Basc / y cv v f
fs / y cv v f
fs / Y
Basc / Y
F2
select all 8 recognizable phenotypes of ♂ progeny, 10 ♂♂ of each kind
y cv v f fs? / Y
+ + + + fs? / Y
y cv v + fs? / Y
+ + + f fs? / Y
y cv + + fs? / Y
+ + v f fs? / Y
y + + + fs? / Y
+ cv v f fs? / Y
Genetically rescuable female sterile
General procedure
mate single ♂♂ x 3 fs ♀♀
mate single ♂♂ x 3 fs / Basc ♀♀
F3
score fertility of each cross
non-Bar ♀♀ x any ♂♂
F4
score fertility of each cross

B. Complementation Mapping

The mutations were also mapped by complementation with stocks bearing duplications and deficiencies in the region y to w. The stocks used and their breakpoints are given in Table 7. Because mie1(1)R1^{o} is not genetically rescuable, it is difficult to test it for complementation with Y-chromosomes to which small portions of the X have been translocated. The ability of the various deficiency or duplication bearing males to rescue embryos produced by mie1(1)R1^{r} homozygous females permitted localization of the mutation to the region of a few bands. mie1(1)R1^{o} was also tested, where possible, by making females heterozygous for a deficiency and mie1(1)R1^{o} and scoring their fertility.

V. COMPLEMENTATION

The test for allelism among female sterile mutants (Figure 4a) consists of making females heterozygous for two female sterile mutations and testing their fertility in crosses with males carrying either mutation. In the special case of a genetically rescuable mutant (Figure 4b) a simpler test may be applied: females homozygous for that mutation are mated with males carrying the mutations to be tested. In both cases (Figures 4a and 4b), sterility of the tested females indicates non-complementation, ie. identity of the mutants.

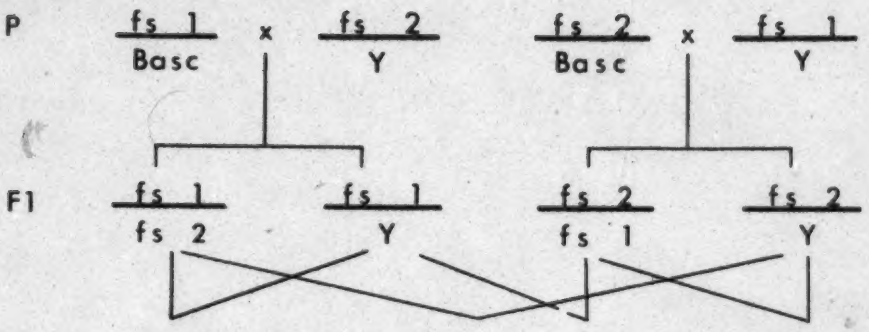
Figure 4. a. General test for allelism of X-linked female sterile mutations.

The crosses are designated according to the following convention: maternally derived chromosomes are placed above paternally derived chromosomes.

b. Test for allelism applicable when one mutation is genetically rescuable.

fs^r = genetically rescuable female sterile mutation.

A



F2 cross 1 cross 2 cross 3 cross 4

no progeny in any of these crosses if fs 1 = fs 2, some progeny in all these crosses if fs 1 ≠ fs 2.

B

F1 fs r / fs if fs r ≠ fs, no progeny if fs r = fs.

VI. CYTOLOGY

The phenotype which distinguishes mie1(1)R1^{r} and mie1(1)R1^{o} from many other maternally influenced embryonic lethal mutations is that many of the embryos produced by homozygous mutant mothers turn brown, that is, they are lethal after blastoderm formation (Würgler, 1968). In this thesis I have referred to brown eggs as post-blastoderm lethal embryos.

It was desired to ascertain whether all the presumptive early deaths might be accounted for by unfertilized eggs. To resolve this question, embryos which had wild-type, mie1(1)R1^{r}, and mie1(1)R1^{o} mothers were collected at various times after a half hour deposition period. They were then dechorionated in 3% calcium hypochlorite for three minutes and processed according to the method of von Borstel and Lindsley (1959). The modified version of Kahle's fixative was employed: distilled water, 37 ml; 95%alcohol 32ml; formalin, 27 ml; glacial acetic acid, 4 ml, and the Feulgen procedure was used for staining. Eggs fixed at 4 - 4½ hours and at 17½ - 18 hours post-deposition were examined using a compound microscope. The criterion for concluding non-fertilization of an egg is that a few (<5) metaphase nuclei which may be polyploid or pycnotic and in various states of degeneration are found in the egg. (von Borstel, 1960).

Embryos produced from the mutant crosses mie1(1)R1^{r}/mie1(1)R1^{r} x mie1(1)R1^{r}/Y and mie1(1)R1^{o}/mie1(1)R1^{o} x mie1(1)R1^{o}/Y were examined for signs of abnormalities at different stages of embryogenesis. These embryos were collected over one-hour periods and aged to $12\pm\frac{1}{2}$, $16\pm\frac{1}{2}$, and $18\frac{1}{2}\pm\frac{1}{2}$ hours. They were then mounted on microscope slides

using double-sided Scotch tape, covered with a drop of immersion oil and a cover glass and then examined under phase contrast at 200X. The immersion oil renders the egg chorion transparent so the embryo itself may be examined. Wild-type embryos treated in this way may be left several hours under the oil and later hatch normally.

VII. MEASUREMENT OF 6-PHOSPHOGLUCONATE DEHYDROGENASE ACTIVITY

The activity of 6-Pgd was assayed in mie1(1)R1^{r}, mie1(1)R1^{o}, wild-type, and Pgd^{-} flies of both sexes using the procedure of Hodgetts (personal communication). Ten adults were weighed, ground in 0.4 ml buffer in 1.0 ml Bellco grinders, then centrifuged in disposable 1 ml conical centrifuge tubes at 8K for ten minutes. The supernatant was removed and stored on ice. The buffer was 0.05 M Tris, pH 7.4 at 23^{0}C which was also 0.01 M in $MgCl_2$.

The reaction mixture analysed consisted of 50λ of the above extract, 50λ of 2 mM NADP in buffer, 50λ of 6-phosphogluconate (6 mM in buffer) and 100λ of buffer. A reference sample for each extract was prepared: the 6-phosphogluconate was omitted and replaced with an additional 50λ of buffer.

Enzyme activity in two replicates of each extract was measured in a Beckman DB - G spectrophotometer by the increase in optical density at 340mu caused by the conversion of NADP to $NADPH^{+}$.

RESULTS

Two mutant stocks (L207 and M163, see Appendix) isolated in the screen for X-linked female sterile mutations showed post-blastoderm embryonic lethality and were chosen for further study. These mutations were designated mie1(1)R1^{r} and mie1(1)R1^{o} respectively, according to the criteria outlined in the Introduction.

L. PRELIMINARY MAPPING OF FEMALE STERILITY

The data shown in Table 3 indicate that both mie1(1)R1^{r} and mie1(1)R1^{o} map in the region between the markers y and cv. In the case of mie1(1)R1^{r} for which data are more numerous, the position is very close to y. Although no accurate map position could be calculated because only a small number of females recombinant for y and cv was tested in each case, mie1(1)R1^{r} and mie1(1)R1^{o} both map quite near y.

II. ALLELISM OF THE TWO MUTANTS

On the basis of similar map positions, mie1(1)R1^{r} and mie1(1)R1^{o} were tested for allelism. Controls showing the non-leakiness of the female sterility phenotypes are given in Table 4, Crosses 1 and 2: mie1(1)R1^{r} and mie1(1)R1^{o} homozygous females are completely sterile when mated to mie1(1)R1^{r} and mie1(1)R1^{o} mutant males respectively. The females heterozygous for these two mutations are sterile when mated with either mutant male (Crosses 3, 4, 5, and 6). This indicates non-complementation of the mutants.

Table 3. Linkage tests of *miel (1) Rl^r* and *miel (1) Rl^o* with *y cv v f*.*

mutant	fertility of recombinant classes								Location of mutant
	y cv v f	*+ + + +*	*y cv v +*	*+ + + f*	*y cv + +*	*+ + v f*	*y + + +*	*+ cv v f*	
miel (1) Rl^r	0-	10-	0-	10-	0-	10-	0-	9-	between *y* & *cv*.
	10+	0+	10+	0+	10+	0+	10+	1+	close to *y*.
miel (1) Rl^o	0-	10-	0-	9-	0-	3-	0-	4-	between *y* & *cv*.
	10+	0+	10+	0+	10+	0+	10+	2+	close to *y*

* + = fertile, - = sterile

see Figure 3 for the details of the mapping procedure.

Table 4. Complementation of the two X - linked miel mutants.

Cross	Parental Genotypes		Number of females tested	Number sterile	Number producing post-blastoderm lethal embryos	Number fertile
	female	male				
1.	$\underline{miel(1)Rl^{r}}/\underline{miel(1)Rl^{r}}$	$\underline{miel(1)Rl^{r}}/\underline{Y}$	41	41	34	0
2.	$\underline{miel(1)Rl^{o}}/\underline{miel(1)Rl^{o}}$	$\underline{miel(1)Rl^{o}}/\underline{Y}$	44	44	15	0
3.	$\underline{miel(1)Rl^{r}}/\underline{miel(1)Rl^{o}}$	$\underline{miel(1)Rl^{r}}/\underline{Y}$	30	30	--	0
4.	$\underline{miel(1)Rl^{r}}/\underline{miel(1)Rl^{o}}$	$\underline{miel(1)Rl^{o}}/\underline{Y}$	30	30	--	0
5.	$\underline{miel(1)Rl^{o}}/\underline{miel(1)Rl^{r}}$	$\underline{miel(1)Rl^{o}}/\underline{Y}$	30	30	--	0
6.	$\underline{miel(1)Rl^{o}}/\underline{miel(1)Rl^{r}}$	$\underline{miel(1)Rl^{r}}/\underline{Y}$	30	30	--	0

Crosses 3 to 6 correspond to Figure 4a, crosses 1 to 4, see footnote Figure 4.

III. GENETIC RESCUABILITY OF *miel(1)Rl^r*

miel(1)Rl^r homozygous females are completely sterile when mated to *miel(1)Rl^r*/Y males (Table 4, Cross 1) and when mated to *miel(1)Rl^o*/Y males (Table 5, Cross 1). However when these females are mated to wild-type males (Table 5, Cross 2), live fertile female offspring are produced. As described in the Introduction this phenomenon of a wild-type allele alleviating maternally effected damage is termed "genetic rescue". This result does not obtain for *miel(1)Rl^o* females (Cross 3).

Genetic rescue in the case of *miel(1)Rl* locus is dominant. Female flies heterozygous for *miel(1)Rl^r* and *miel(1)Rl^o* when mated with *miel(1)Rl$^+$*, *w sn^3*/Y males produce daughters as do *miel(1)Rl^r* homozygous females (Crosses 4 and 5). In these crosses all the males produced were found to have resulted from non-disjunction at the second meiotic division of the oocyte. This was established by the finding that they were patroclinous (*w sn^3*).

IV. PRECISE MAPPING OF THE *miel(1)Rl* LOCUS

A. Recombination Mapping

Since *miel(1)Rl^r* and *miel(1)Rl^o* are alleles, the data from Table 3 were pooled for a calculation of a very approximate location of the locus.

Table 5. The phenotype of genetic rescue.

Cross	Parental Genotypes		Number of females tested	Number* fertilized	Number sterile	Number fertile	Progeny
	female	male					
1.	$\underline{miel(1)Rl^{r}}/\underline{miel(1)Rl^{r}}$	$\underline{miel(1)Rl^{o}}/\underline{Y}$	30	--	30	0	
2.	$\underline{miel(1)Rl^{r}}/\underline{miel(1)Rl^{r}}$	$+/\underline{Y}$	37	31	0	31	all female
3.	$\underline{miel(1)Rl^{o}}/\underline{miel(1)Rl^{o}}$	$+/\underline{Y}$	30	6	6	0	
4.	$\underline{miel(1)Rl^{r}}/\underline{miel(1)Rl^{o}}$	$\underline{w\ sn^{3}}/\underline{Y}$	115	111	0	111	3331 females 7 $\underline{w\ sn^{3}}$ males
5.	$\underline{miel(1)Rl^{o}}/\underline{miel(1)Rl^{r}}$	$\underline{w\ sn^{3}}/\underline{Y}$	114	105	0	105	2514 females 4 $\underline{w\ sn^{3}}$ males

* fertilized females were identified by the criterion that some post-blastoderm lethal embryos were produced, see p.

y	miel(1)R1$^+$	+	20
y	miel(1)R1	+	0
+	miel(1)R1$^+$	cv	3
+	miel(1)R1	cv	13
Total			36

The miel(1)R1 locus is 3/36 or 0.08 of the distance from y to cv (13.5 map units apart as cited in Lindsley and Grell, 1968).

On the basis of the preliminary mapping, miel(1)R1^r was remapped using the short method (see Materials and Methods) and the outside markers y and w. The results of these experiments are given in Table 6.

Using data from experiment 1, the distance between y and w was calculated as 0.68 map units. This is less than half the published distance of 1.5 map units (lindsley and Grell, 1968). Therefore in mapping miel(1)R1^r it was necessary to assume that the observed decrease in recombination is uniform over the total distance between y and w, that is, the decrease is not localized to a few small intervals within the region.

Data from both experiments were pooled for the estimation of the location of miel(1)R1^r.

y	miel(1)R1$^+$	+	3
y	miel(1)R1^r	+	4
+	miel(1)R1$^+$	w	1
+	miel(1)R1^r	w	8
Total			16

Table 6. The location of *miel(1)R1^r* by recombination with a chromosome bearing the markers *yellow* and *white*.

Recombinant Class	Experiment 1					Experiment 2			
	Number recovered	Number tested	Number mated	Sterile	Fertile	Number tested	Number mated	Sterile	Fertile
y w	1400	40	12	0	12	32	5	0	5
+ +	1236	40	40	40	0	26	9	9	0
y +	9	8	6	3	3	7	1	1	0
+ w	9	9	8	7	1	1	1	1	0

Refer to Figure 3 for the crosses which produce these recombinant males. They were tested by mating to *miel(1)R1^r/miel(1)R1^r* females.

The estimated map position and its 95% confidence interval were calculated using the formulae used by Nash (1963, Table 9.2b).

$Z + \frac{a}{a+b} \cdot x$ gives the estimated map position of $\underline{miel(1)R1}^r$ where x is 1.5 map units, the published distance between the markers y and w, while Z is 0.0, the map position of y, a and b are the numbers of recombinational events between y and $\underline{miel(1)R1}^r$, and between $\underline{miel(1)R1}^r$ and w, respectively. From the data it is seen that a = 5, b = 11 and the calculated map position of $\underline{miel/R1}^r$ is therefore 0.47.

The 95% confidence interval for this map position is calculated from the equation: location = Z + p.x where p represents the roots of the following equation

$$p^2((a + b)^2 + \chi_1^2 (a + b)) - p(2a^2 + 2ab + \chi_1^2 (a + b)) + a^2 = 0$$

χ_1^2 is that value of χ^2 where the probability = 0.05, with one degree of freedom.

Thus the map position of $\underline{miel(1)R1}^r$ lies between 0.21 and 0.83 map units as analysed by recombination at the 95% level of confidence.

B. Complementation Mapping

Mapping of the $\underline{miel(1)R1}$ locus using duplications and deficiencies extending into the y - w region was carried out in an effort to improve upon the recombination analysis above.

Tables 7 and 8 and Figure 5 are pertinent to the analysis of the complementation tests of $\underline{miel(1)R1}^r$ and $\underline{miel(1)R1}^o$ with duplication and deficiency bearing stocks. Table 7 gives the cytological breakpoints of the duplication and deficiency chromosomes. Table 8 gives the results of the complementation tests. The cross $\underline{miel(1)R1}^r$/ $\underline{miel(1)R1}^r$ x y sc $\underline{gy}^{X11}$/$\underline{w}^+.Y$ males (Cross 1) yielded essentially equal numbers of male and female progeny. This is the result expected if

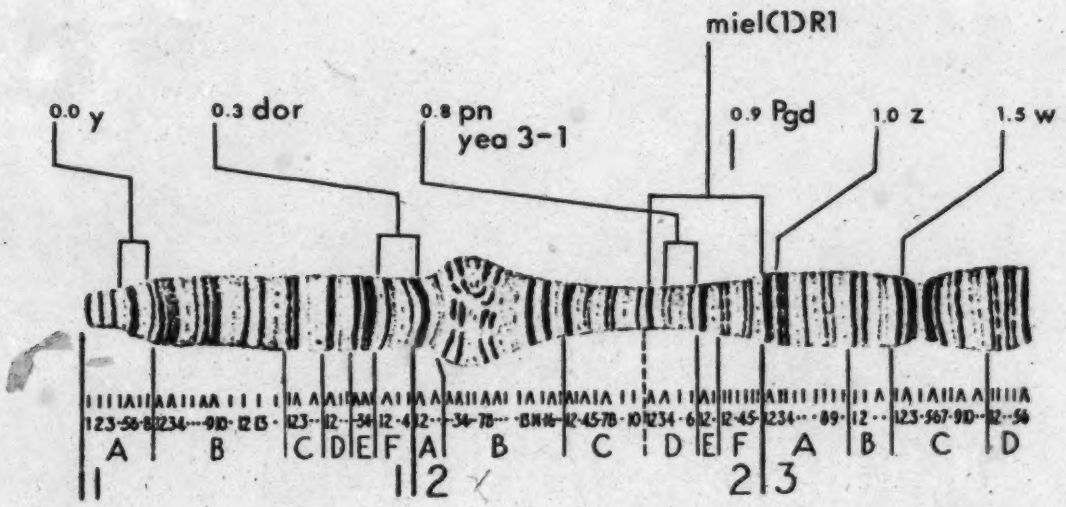
Table 7. Cytological breakpoints of deficiency and duplication bearing chromosomes.

Deficiencies	Cytological Breakpoints	Reference
Df(1)X12	2F6-3A1; 3B5-3C1	Judd et al 1972.
$Df(1)w^{rJ1}$	3A1-2; 3A4-6	Judd et al 1972.
Duplication		
$w^{+}.Y$	2D1-2; 3D4-5	Judd et al 1972.

Table 8. Complementation of miel(1)R1^{r} and miel(1)R1^{o} with duplications and deficiencies in the region yellow to white.

Cross	Parental Genotypes			Progeny		
	female		male	female	male	total
1.	miel(1)R1^{r}/miel(1)R1^{r}	x	y sc gt^{X11}/w^{+}.Y	40	37	77
2.	miel(1)R1^{r}/miel(1)R1^{r}	x	Df(1)w^{rJ1}/w^{+}.Y	50	49	99
3a	miel(1)R1^{r}/Df(1)X12	x	miel(1)R1^{r}/Y	352	179	531
3b	miel(1)R1^{r}/Df(1)X12	x	miel(1)R1^{r}/w^{+}.Y	285	105 y 159 +	549
4a	miel(1)R1^{o}/Df(1)X12	x	miel(1)R1^{o}/Y	471	239	710
4b	miel(1)R1^{o}/Df(1)X12	x	miel(1)R1^{o}/w^{+}.Y	365	165 y 216 +	746

Figure 5. A segment of the X-chromosome showing the location of the miel(1)R1 locus, the cytological extent of the duplication and deficiencies used to establish its position, and the position of other relevant markers.



Df(1)X12

Df(1)w^{rJ1}

w^{+} . Y

miel(1)R1 is located in the w^+.Y duplication. This result localizes miel(1)R1 to the region between salivary gland chromosome bands 2D1-2 and 3D4-5.

Crosses 3a and b show that miel(1)$R1^r$ is not located in Df(1)X12 which covers the region 2F6-3A1 to 3B5-3C1. Crosses 4a and b confirm this result for miel(1)$R1^o$. These results further restrict the location of the miel(1)R1 locus within the region 2D1-2 to 3D4-5 to those bands which are distal to 3A1 or proximal to 3B5.

Finally the cross miel(1)$R1^r$/miel(1)$R1^r$ x Df(1)w^{rJ1}/Y (Cross 2) yielded both males and females as would be expected if the miel(1)R1 locus were not included in the deficiency. This result eliminates the band 3C1 from consideration. Since w is located in band 3C2, and miel(1)$R1^r$ has been mapped distal to w, bands 3C3 to 3D4 cannot include the location of miel(1)R1.

Thus the miel(1)R1 locus maps between salivary bands 2D1-2 and 2F6-3A1, a region of some fourteen bands, as shown in Figure 5.

C. Salivary Gland Chromosome Analysis

Salivary glands were dissected from female third instar larvae produced in the cross miel(1)$R1^o$ /FM7b x $\overline{XY}$. Those of a larva presumably of the miel(1)$R1^o$/$\overline{XY}$ genotype (the X-chromosome lacked the inversions present in FM7b) were examined for the possible presence of a large rearrangement or deficiency in the 2D1-2; 2F6-3A1 region with which the mutant phenotype might be associated. No evidence of heterozygosity was found in this region. Thus if miel(1)$R1^o$ is a

deficiency, it must be a small one; probably a band or less. Since mie1(1)R1^{r} has a less extreme phenotype, it was considered unlikely that it would be a deletion.

V. VIABILITY PATTERNS OF THE MUTANTS

The results reported in this section are all from experiments conducted in the following way. Females to be tested were collected as virgins and aged three days. They were then premated for one day with appropriate males which had also been aged three days. The experiments consisted of collecting the eggs laid by individual females for four consecutive twenty-four hour periods following the premating. Eggs were counted immediately following each twenty-four hour laying period. These eggs were then rescored twenty-four hours later for hatch and post-blastoderm embryonic lethality.

The numbers of eggs reported in the crosses in Table 9a are corrected to eliminate as far as possible unfertilized eggs. Each number includes all eggs laid on a given day by a female which produced post-blastoderm lethal embryos and/or larvae on that day.

A. Viability and fertility of heterozygous females

A recessive lethal component of phenotype is often associated with mutations affecting fertility. For example, this is obvious from previous studies on dor (Bischoff and Lucchesi, 1971) and from the data on dor included in Table 10, Crosses 4,5,6, and 7. In order

to determine the viability of the two alleles at the miel(1)R1 locus, crosses yielding both heterozygous and homozygous mutant individuals were scored for survival at various stages of development. These results are shown in Tables 9a and 9b. Cross 1 in both tables is a control where + is the Amherst wild-type X-chromosome. Crosses 2 and 5 are matings of heterozygous miel(1)R1^{o} and miel(1)R1^{r} females and their respective mutant males.

i) miel(1)R1^{o}

For the cross between heterozygous miel(1)R1^{o} females and mutant males (Cross 2) three main points can be made. First, there is high viability at each developmental stage, comparable to that of the control flies (Table 9a, Cross 1). Second, there were fewer homozygous female progeny produced than expected: they were rescued .68 as often as heterozygous female progeny. (Table 9b, Cross 1) However mutant males produced in this cross were recovered in approximately equal numbers to heterozygous females. Third, the low level of post-blastoderm embryonic lethality (1.2%) is significantly higher than that obtained for the control cross (0.2%) ($\chi^2_1 = 7.1$, $p = 0.01$).

ii) miel(1)R1^{r}

Progeny of miel(1)R1^{r} heterozygotes (Table 9a, Cross 5) also have high viability at each stage. The progeny resulting from this cross are recovered in close to the expected proportions (Table 9b, Cross 5). This cross also shows a very low level of late embryonic lethality (0.6%), but again higher than control.

B. The conditional nature of embryonic lethality

i) miel(1)R1^{o}

In experimental matings of homozygous miel(1)R1^{o} females with

Table 9a. Survival at different developmental stages of progeny of heterozygous and homozygous *miel(1)Rl^{o}* and *miel(1)Rl^{r}* mutant females.

Cross	Parental Genotypes		Number of Eggs	Survival Number and (Percent of Eggs Scored)			
	female	male		Late Lethal Embryos	Larvae	Pupae	Adults
1.	+/Basc	+/Y	1695	4 (0.2)	1591 (93.8)	1554 (91.7)	1463 (86.4)
2.	miel(1)Rl^{o}/Basc	miel(1)Rl^{o}/Y	2420	28 (1.2)	2227 (92.1)	2165 (89.5)	2095 (86.5)
3 a.	miel(1)Rl^{o}/miel(1)Rl^{o}	miel(1)Rl^{o}/Y	451	169 (37.5)	0		
b.			380	147 (38.7)	0		
4 a.	miel(1)Rl^{o}/miel(1)Rl^{o}	+/Y	277	82 (29.6)	0		
b.			774	288 (38.4)	0		
5.	miel(1)Rl^{r}/Basc	miel(1)Rl^{r}/Y	1857	11 (0.6)	1642 (89.5)	1570 (84.7	1554 (83.3)
6 a.	miel(1)Rl^{r}/miel(1)Rl^{r}	miel(1)Rl^{r}/Y	557	458 (79.4)	0		
b.			2323	1902 (81.9	0		
7 a.	miel(1)Rl^{r}/miel(1)Rl^{r}	+/Y	455	184 (40.4)	166 (36.5)	142 (31.2)	115 (25.3) females
b.			774	288 (37.2)	307 (39.7)	237 (30.4)	227 (29.4) females
c.			2393	870 (36.4)	644 (26.9)	---	--- females

Table 9b. Distribution of progeny produced by heterozygous miel(1)R1^{o} and miel(1)R1^{r} females.

Cross	Parental Genotypes		Adult Progeny	Numbers and frequencies of Progeny genotypes			
	female	male					
1.	+/Basc	+/Y	1463	+/+ 394 (.95)	+/Basc 414 (1.0)	+/Y 370 (.90)	Basc/Y 214 (.69)
				miel(1)R1^{o}/miel(1)R1^{o}	miel(1)R1^{o}/Basc	miel(1)R1^{o}/Y	Basc/Y
2.	miel(1)R1^{o}/Basc	miel(1)R1^{o}/Y	341	65 (.68)	96 (1.0)	104 (1.08)	76 (.79)
				miel(1)R1^{r}/miel(1)R1^{r}	miel(1)R1^{r}/Basc	meil(1)R1^{r}/Y	Basc/Y
5.	miel(1)R1^{r}/Basc	miel(1)R1^{r}/Y	1174	321 (1.04)	310 (1.0)	287 (.93)	256 (.83)

* frequency is given relative to Basc heterozygote survival.

miel(1)Rl^{o}/Y or +/Y males (Table 9a, Crosses 3 and 4 respectively) conducted at different times, no larvae hatched from a total of 1882 eggs. In both crosses very similar fractions of the eggs laid develop past the blastoderm stage and then die; the range is 30 to 39% for the four experiments. These data confirm and extend the result of similar crosses reported in Table 4, Cross 2 and Table 5, Cross 3.

ii) miel(1)Rl^{r}

In crosses of homozygous miel(1)Rl^{r} females with miel(1)Rl^{r}/Y males (Table 9a, Cross 6), no larvae hatched from a total of 2880 eggs. However, when these homozygous miel(1)Rl^{r} females were mated with +/Y males (Cross 7), adult progeny were produced. As in a previous cross (Table 5, Cross 2) these adults were all female. The total number of post-blastoderm lethal embryos (1342) is significantly greater than larvae hatched (1118) $\chi^2_1 = 21.1$, $P<.001$ and most of the difference is in experiment c. The total fraction of post-blastoderm lethal embryos plus hatched larvae in Cross 7 (.69) is lower than the fraction of post-blastoderm lethal embryos (.81) in Cross 6 where no rescue was observed, but if experiment c is excluded, this fraction rises to (.77) and the compared fractions are very similar, as expected.

Survival of rescued larvae to adulthood is not as high as survival of larvae to adulthood when miel(1)Rl^{r} heterozygotes were tested as female parents (Cross 5): approximately 83% of the rescued larvae survived compared to 94% survival of offspring of heterozygous females.

VI. DEVELOPMENTAL STUDIES ON LETHAL EMBRYOS

Lethal embryos for these studies were collected from the crosses $\underline{miel(1)R1^{r}}/\underline{miel(1)R1^{r}}$ x $\underline{miel(1)R1^{r}}/\underline{Y}$ and $\underline{miel(1)R1^{o}}/\underline{miel(1)R1^{o}}$ a $\underline{miel(1)R1^{o}}/\underline{Y}$. Control (non-lethal embryos were collected from crosses of Amherst wild-type males and females. The parents used for these experiments were fed live yeast prior to the collection of eggs.

Eggs collected during half-hour laying periods were aged four hours and then divided into two samples. Embryos from the first sample were fixed, whole-mounted, and stained as described in the Materials and Methods. Embryos from the second sample were aged a further twenty-four hours and then scored to ascertain the fraction of white pre-blastoderm lethal embryos. Among the whole-mounted and stained embryos 2/20 (.10) Amherst, 6/24 (.25) $\underline{miel(1)R1^{r}}$ and 4/18 (.22) $\underline{miel(1)R1^{o}}$ failed to take up stain visible to the eye.

These non-visibly stained embryos were examined under high magnification and oil immersion and scored for the numbers of nuclei to determine whether they had in fact been fertilized. One egg contained eight Feulgen stained bodies which appeared to be either degenerating nuclei or parts of nuclei. The rest of the eggs contained either one or two nuclei showing basket or pycnotic metaphase configurations of chromosomes.

The fraction of pre-blastoderm embryonic lethal (white eggs) in the aged sample was 4/50 (.08) for Amherst controls, 16/48 (.33) for $\underline{miel(1)R1^{r}}$ and 6/18 (.33) for $\underline{miel(1)R1^{o}}$; that is, comparable to the fraction of eggs scored as unfertilized in each case above.

These findings are consistent with the interpretation that all of the white eggs were unfertilized (von Borstel, 1960; Doane, 1960).

This result is important both to the analysis of the data in the tables, and to the establishment of an important aspect of the phenotypes of the mutants themselves, namely that in neither mie1(1)R1^{r} nor mie1(1)R1^{o} does there appear to be any substantial component of maternally effected pre-blastoderm mortality.

When the visibly stained 4 - 4½ hour-old embryos were examined microsopically, they were found to be in various stages of gastrulation. The ventral furrow was found to be a prominent feature of these embryos (see Table 1). As can be readily seen from the data presented above, the number of brown embryos in the aged sample was similar to the number of gastrulae in the egg sample fixed four hours after deposition. This finding is corroborating evidence that all mutant embryos which are fertilized reach the gastrula stage and will be recognizable as such by the fact that they turn brown.

Substantial tissue differentiation had occurred in a sample of visibly stained 18 - 18½ hour-old embryos, but because of the many stained nuclei, recognizable structures other than gut were not easily discerned. Consequently, the examination of unstained embryos seemed warranted.

Live, unstained twelve hour-old embryos of both mie1(1)R1^{r} and mie1(1)R1^{o} showed evidence of tissue differentiation. Examination of older embryos revealed that mie1(1)R1^{o} embryos are more severely affected than those of mie1(1)R1^{r}. In sixteen hour-old mie1(1)R1^{r} embryos gut differentiation looked normal; malpighian tubules could be distinguished and structures which appeared to be mouth hooks were visible. Segmentation appeared normal in the posterior 2/3 of the embryo, especially ventrally.

However the anterior dorsal region appeared unsegmented and disorganized. Muscular contraction was observed. Some gut differentiation was apparent in miel(1)R1^{o} embryos of the same age. Segmentation in the posterior one-third to one-half of the embryo looked reasonably normal. However, the anterior part of the embryo showed no segmentation and appeared very disorganized. Some contractions were observed in what appeared to be a pouch of gut forced to the anterior in one embryo.

Embryos which were eighteen hours-old were also examined. These embryos had been covered with less immersion oil and subsequently burst anteriorly. Cells and tissues then flowed out and examination was easier than in unburst embryos. Gut, tracheae, nervous tissue and a tissue which may have been either salivary gland or fat body because of its large nuclei were all observed in burst miel(1)R1^{r} embryos. miel(1)R1^{o} embryos had gut, but none of the other tissues mentioned above was observed. There were many lumps of cells which could not be distinguished as particular tissues. Large numbers of what appeared to be yolk granules were present.

VII. INTERACTIONS OF miel(1)R1^{r} AND miel(1)R1^{o} WITH OTHER MUTATIONS

Various mutations mapping within the regions in which miel(1)R1 was located were examined for allelism or other interactions with miel(1)R1^{r} and miel(1)R1^{o}. These were dor: 1-0.3; a locus which has genetically rescuable miel alleles, yea 3-1: 1-0.8; a yeast requiring auxotrophic mutant isolated by Falk (1973) which is also a recessive female semi-sterile, and Pgd^{-}: 1-0.9; a null allele of the 6-phosphogluconate dehydrogenase locus. Maternally transmitted Pgd is known to be active in wild-type embryos from the study of Wright and Shaw (1970).

A. deep orange

The female sterile dor allele used in these experiments behaves as a non-rescuable mutant under the conditions of the experiment (see Table 10, Cross 1). The males of the stock are fertile (Cross 2). An X-bearing sperm from a dor/Y male will rescue embryos produced by miel(1)R1^{r} mothers (Cross 3). Thus miel(1)R1^{r} and dor are not allelic. The other crosses confirm this conculsion. In the crosses where all four genotypes of progeny could be distinguished (Crosses 6 and 7) there was found to be a deficit of miel(1)R1^{r}/Y males relative to miel(1)R1^{r} heterozygotes that was not seen in miel(1)R1^{r}/Basc x miel(1)R1^{r}/Y crosses (Table 9b, Cross 5).

B. yeast 3-1

The only crosses involving yea 3-1 that were tested were miel(1)R1^{o}/yea3-1 x miel(1)R1^{o}/Y and miel(1)R1^{o}/yea 3-1 x yea 3-1/Y. In the first cross, twenty females were tested of which eleven were sterile and nine fertile over an eight day period. Nine of the sterile females survived and were remated with Oregon-R wild-type males. Two of these females subsequently became fertile. Of 304 progeny produced in this cross, there were 170 females and 134 males.

In the second cross, miel(1)R1^{o}/yea 3-1 x yea 3-1/Y, twelve of fifteen tested females were fertile and at least four of these females produced many post-blastoderm lethal embryos. The rest of the females and those females which were fertile in the first cross could not be checked for the presence of post-blastoderm lethal embryos as the food had been well masticated by larvae before vials were scored. In this cross the sex ratio was 46 females to 24 males.

C. 6-Phosphogluconate dehydrogenase

The results of the 6-phosphogluconate dehydrogenase assays

Table 10. Complementation of *miel(1)R1^r* with *dor*.

Cross	Parental Genotypes		Progeny Genotypes				
	female	male	+/+	+/dor	+/Y	dor/Y	
1.	dor/dor	+/Y		0		0	
2.	+/+	dor/Y		+	+		
			miel(1)R1^r/miel(1)R1^r	miel(1)R1^r/dor	miel(1)R1^r/Y	dor/Y	
3.	miel(1)R1^r/miel(1)R1^r	dor/Y		78		0	
4.	miel(1)R1^r/dor	miel(1)R1^r/Y	282			139	7
5.	dor/miel(1)R1^r	miel(1)R1^r/Y	322			132	13
			dor/dor	miel(1)R1^r/dor	miel(1)R1^r/Y	dor/Y	
6.	miel(1)R1^r/dor	dor/Y	1	45	14	7	
7.	dor/miel(1)R1^r	dor/Y	10	170	146	16	
8.	dor^1/miel(1)R1^r	miel(1)R1^r/Y	636		319	0	

are shown in Table 11. The given values are averages of two replicates. The data show that neither mie1(1)R1^{r} nor mie1(1)R1^{o} is a Pgd^{-} allele; niether is yea 3-1. Although mie1(1)R1^{r} females appear to have decreased Pgd activity and both sexes of mie1(1)R1^{o} show increased levels of enzyme activity, the significance, if any, of these results to the mie1(1)R1 mutant phenotypes is unknown.

Table 11. 6-phosphogluconate dehydrogenase activity in male and female Drosphilia.

(Δ OD, 340 / min/ mg wet wt. x 10^{-2})

Genotype	Female		Male
	Experiment 1	Experiment 2	Experiment 2
Pgd^{-}	___	0.06	0.3
Amherst	2.2	2.6	3.0
miel(1)R1^{r}	1.7	1.6	3.4
miel(1)R1^{o}	3.8	2.6	4.2
yea 3-1	___	___	3.2

DISCUSSION AND CONCLUSIONS

I. THE PHENOTYPES OF *miel(1)R1*^{r} AND *miel(1)R1*^{o}

A. Gene Localization

A new locus, *miel(1)R1*, which has a maternal effect on embryogenesis has been identified. Two mutants at this locus have been isolated and designated *miel(1)R1*^{r} and *miel(1)R1*^{o}. The locus maps between 0.21 and 0.83 on the X-chromosome at the 95% level of confidence. The locus has also been mapped cytologically to the salivary gland chromosome bands between 2D1 and 3A1, a region of some fourteen bands. Since Lindsley and Grell (1968) catalogue no small deficiencies in the 2D1 - 2A1 region, a more precise localization is not presentl possible.

B. Female Fertility and Genetic Rescue

One of the mutations, *miel(1)R1*^{r} is genetically rescuable. The other mutation, *miel(1)R1*^{o}, is more severe and is not genetically rescuable. When females were made heterozygous for the two mutations and mated with wild-type males, it was found that the property of genetic rescue is at least partially dominant at this locus. Since no attempt was made to count embryos or observe them cytologically, it remains unknown whether the phenotype of the double heterozygote is intermediate in severity between *miel(1)R1*^{r} and *miel(1)R1*^{o}, as might be expected.

These mutations cause complete female sterility since no larvae ever hatch in crosses of homozygous *miel(1)R1*^{o} and *miel(1)R1*^{r} females to *miel(1)R1*^{o}/Y or *miel(1)R1*^{r}/Y males. Essentially the same

conclusion may be drawn from the matings of mie1(1)Rl^{r} and mie1(1)Rl^{o} homozygous females with wild-type males. In the case of mie1(1)Rl^{r}, although heterozygous females are rescued, no male "escapers" were ever observed. The only males produced were found in the mie1(1)Rl^{r}/mie1(1)Rl^{o} and mie1(1)Rl^{o}/mie1(1)Rl^{r} heterozygote crosses with +/Y males and they were shown genetically to have been non-disjunctional patroclinous males carrying a mie1(1)Rl^{+} allele.

It appears that whatever the product of mie1(1)Rl^{+}, it must be present at a sufficient concentration in the embryo, either as a component of the egg cytoplasm derived from the mother, or as a product of the embryonic genome, if embryogenesis is to be completed. We know from mie1(1)Rl^{r} that the product of the wild-type paternally contributed allele can act in mutant embryos some time after blastoderm formation. The lack of rescue in the case of embryos which had mie1(1)Rl^{o} mothers might be attributed to the severity of the mutant effect. It is possible that damage prior to possible action of a wild-type paternally contributed allele is so severe that this action can not alleviate the effect of the mutant cytoplasm. It is also possible that in the case of mie1(1)Rl^{o}, the + allele contributed by the male parent fails to act, hence embryos die. In turn, it might be that this failure to act is in some way related to the severity of the defect in the mutant mie1(1)Rl^{o} cytoplasm.

The rescue of embryos which are genetically capable of being rescued (that is, they had mie1(1)Rl^{r}/mie1(1)Rl^{r} homozygous mothers and

received a wild-type allele from their fathers) appears not to be complete. Assuming that random fertilization of embryos with X- and Y-bearing sperm takes place, and also that all embryos receiving the mie1(1)R1^{+}, X-bearing sperm survive to hatch, 50% of the fertilized eggs should hatch. There were three experiments in which genetic rescue was observed and larvae counted (Table 9a, Cross 7). The number of larvae hatching (1118) was significantly less than the number of post-blastoderm lethal embryos (1346), $\chi^2_1 = 21.1$, $P < .001$. If no male larvae hatch, half the excess of post-blastoderm lethal embryos over hatched female larvae corresponds to heterozygous female embryos in which the action of a wild-type allele failed to compensate for the defect in the egg cytoplasm. From pooled data (Table 9a, Cross 7) it is seen that 10% of female embryos did not hatch.

It is possible that in these rescuable but non-rescued embryos the + allele failed to be turned on. Alternatively, these embryos were already very "sick" before the wild-type allele could act - possibly some mutant egg cytoplasms are more defective than others. Such "sick" embryos might approach the usual condition of lethal mie1(1)R1^{o} embryos. At present it is not possible to distinguish between these alternatives.

The notion that heterozygous rescuable embryos are sick is consistent with observations that the percentage of mie1(1)R1^{r}/+ embryos from mie1(1)R1^{r} mothers reaching the pupal (85.6% of larvae) and adult (81.1%) of pupae) stages is much smaller than in the heterozygote control cross (Table 9a, Cross 5) where 95.7% of larvae pupated

and 98.4% of pupae eclosed. This mortality of the rescued zygotes might also be interpreted as being due to a residual effect of having had an abnormal cytoplasm as an oocyte and as an embryo. The similarity in frequencies of post-blastoderm embryonic lethality in the crosses of homozygous mie1(1)R1^{o} females with mie1(1)R1^{o}/Y males (Table 9a, Cross 3) and with +/Y males (Cross 4) indicated that there is no conditional pre-blastoderm lethality which can be postponed until a later embryonic stage by the action of a wild-type allele. This finding is consistent with the notion that embryonic genes do not act until blastoderm formation. In crosses to mutant males the average frequency of embryos from mie1(1)R1^{o} mothers reaching post-blastoderm stages before dying (36.7%) (Table 9a, Cross 3) is about half that (81.4%) of those produced by mie1(1)R1^{r} mothers (Cross 6). It is therefore probable that many of the embryos produced by mie1(1)R1^{o} mothers die earlier than those produced by mie1(1)R1^{r} mothers. The only other data bearing on this point are derived from the experiment in which embryos were collected from yeast-fed mutant females and studied in whole mounts to determine whether early deaths could all be attributed to non-fertilization. Although the small sample size limits the value of these data, the frequency of post-blastoderm death of mie1(1)R1^{o} embryos (72%) was double that produced by females fed on medium with a lower yeast concentration, suggesting that at least a sizeable proportion of the preblastoderm lethality mentioned above occurs in fertilized eggs. The possibility remains that both the comparisons of post-blastoderm lethality in the two mutants and of mie1(1)R1^{r} in two nutritional regimes can be explained by differences in fertility rates.

The rescue of male embryos in the cross mie1(1)R1^{r}/mie1(1)R1^{r} x y sc gt^{X11}/w^{+}.Y shows that genetic rescue is not a sex limited phenomenon such as occurs for the mutant snl Collaianne and Bell, 1972). Baker (1973) has also found this result for cin.

In addition to the main effects of the mutations certain aspects of their phenotypes as heterozygous females warrant attention. The test mating of mie1(1)R1^{o}/Basc produced fewer female offspring (Table 9a, Cross 2) than was expected, although it produced the expected number of mie1(1)R1^{o}/Y offspring. It is not possible to distinguish between recessive semi-lethality and semi-dominance of the female sterility as the source of this sex limited viability effect. However, although one might expect that if semi-dominance of female sterility were the correct explanation, mie1(1)R1^{o}/Y males would also be reduced relative to heterozygous females, the precedent of the da and abo mutants (Sandler, 1970; 1972) leads to some caution in making the interpretation. Semi-dominance of female sterility might be invoked to explain the significant increase in post-blastoderm embryonic lethality observed in the mie1(1)R1^{o} heterozygote cross (Table 9a, Cross 2) over that in the control (+/Basc x +/Y) cross (Cross 1). This embryonic lethality was not sufficient to account for all of the reduction of mie1(1)R1^{o}/mie1(1)R1^{o} progeny from expected. In contrast to mie1(1)R1^{o}, it appears that there is no inviability associated with the mie1(1)R1^{r} homozygous female genotype. However an increase in post-blastoderm embryonic lethality was observed: it was at a level intermediate between that observed for mie1(1)R1^{o} and for the control cross. This finding is consistent with the interpretation that mie1(1)R1^{r} is a less severe

(damaging) mutation than miel(1)R1^{o} may not be associated with the sex-limited inviability effect.

Occasionally, defects in abdominal segmentation and unilateral wing defects were observed in both heterozygous and homozygous flies of either mutant genotype. These are occasionally seen in the Amherst wild-type stock, but have never been observed in the rescued females produced by doubly heterozygous mutant mothers which would be expected to be the most severely affected rescued individuals. Thus, it does not seem probable that these defects relate to the mutant phenotypes. These defects occurred at very high frequency in rescued amx (Shannon, 1972b) and fu (Counce, 1956b) females.

C. Embryonic Lethality

The greater extent of embryonic abnormalities observed in lethal miel(1)R1^{o} embryos than in miel(1)R1^{r} embryos is consistent with the overall greater severity of mutant effects in miel(1)R1^{o}. In both mutants, embryonic development was relatively more normal at the posterior end than anteriorly. In rescued miel(1)R1^{r}/+ females, however, the external morphology of the adult head shows no defects. It may be that normalcy of head structures is an absolute prerequisite for survival to adulthood in these mutants. Perhaps those individuals that die during the larval and pupal stages die for this reason. A similar kind of conclusion has been drawn by Shannon (1972a) with respect to amx, and by Fausto-Sterling (1971a) with respect to r and fu from her studies on mosaics.

Hartwell et al. (1970) and Suzuki (1970) have elegantly demonstrated in their studies of temperature sensitive lethal mutations in yeast and in Drosophila, that the time during development at which

a gene product may be required ("execution point" or "temperature sensitive period") may differ greatly from the time at which the expression of phenotype ("termination point" or "lethal phase") occurs. These times may also differ from the time of gene action, but their studies are less revealing on this point.

There is presently no accurate information about the time(s) of death of the embryos produced by homozygous mel(1)R1^{r} and mel(1)R1^{o} females, or about the time when abnormalities in their development are first detectable. This latter time would give an estimate for the latest possible execution point or period of the mutation. By inference, this time might be before blastoderm formation because many embryos produced by mel(1)R1^{o} mothers appear to die prior to this time. It is also probable that in some embryos a second execution period occurs after gastrulation, because some mutant mel(1)R1^{o} and most mutant mel(1)R1^{r} embryos die subsequent to gastrulation, and also because mel(1)R1^{r} embryos are rescuable by post-blastoderm wild-type gene activity. The manifestation of the defects of the mutations, (their termination points) will depend on both the execution period (s) and also the severity of the mutant effects.

The dominance relationship mel(1)R1^{+} > mel(1)R1^{r} > mel(1)R1^{o} would suggest that at least mel(1)R1^{r} is a mutation of the hypomorph type (Muller, see Hadorn, 1961). Hadorn has suggested that if a mutation is hypomorphic compared to its wild-type allele, viability might depend on physiological reactions which require a definite threshhold. A substance which is essential for development might be altered or produced in insufficient amounts. The only circumstance

under which mie1(1)R1^{o} mutant embryos have been shown to survive occurs when their maternally derived cytoplasm has been derived from heterozygous females: therefore it is possible that mie1(1)R1^{o} is an amorph, although the possibility also exists that it is a more extreme hypomorph than mie1(1)R1^{r}.

A time of gene action must occur during oogenesis since mie1(1)R1^{r} and mie1(1)R1^{o} are maternally influenced embryonic lethal mutants, and another may occur during post-blastoderm embryogenesis, since one of the mutants is genetically rescuable. The data on incomplete rescue of rescuable embryos indicate that the action of the wild-type allele is more effective when expressed during oogenesis than when expressed solely during embryogenesis. This was the conclusion reached by Shannon (1972b) for amx in which only slight genetic rescue occurs.

The mie1(1)R1^{+} product may also act during other developmental stages since homozygous mie1(1)R1^{o} females are significantly less viable than their heterozygous sisters.

It is possible that the mie1(1)R1^{r} and mie1(1)R1^{o} mutations exert their severest effects during embryogenesis simply because the embryo is a closed nutritional system, and that gene product is required continuously or at many times during post-embryonic development. However, on this supposition the mutations show relatively little effect in other stages, except for the previously mentioned effect on progeny of heterozygous mie1(1)R1^{o}/Basc females, because these stages are not closed and feeding might alleviate the mutant effects. More sophisticated experimental approaches are required to resolve these questions

of whether gene action is continuous or temporally limited and whether the embryo exhibits a specific sensitivity to deficiencies of the required product.

II. INTERACTIONS OF *mel(1)R1*^{r} AND *mel(1)R1*^{o} WITH OTHER MUTATIONS

A. A Possible Interaction with *deep orange*.

Although the *mel(1)R1* and *dor* loci are not the same, it is possible that products of these mutant loci do interact to cause embryonic lethality. This is suggested by the reduced numbers of *mel(1)R1*^{r} males produced by females heterozygous for *dor* and *mel(1)R1*^{r} mated with *dor*/Y males (Table 10, Crosses 6 and 7). Although the data is scanty, were it to prove a real effect, one might postulate that *dor* and *mel(1)R1* mutant products interact in the cytoplasm of an egg. In the absence of biochemical evidence, it is not possible to distinguish whether this interaction is one of function; that is, the wild-type gene products act together in some process, or the damages caused separately by *dor* (cf its recessive semi-lethality) and *mel(1)R1* mutations are additive. In this context, one would like to examine the embryos produced by a female which is a double homozygote for the two mutations.

B. Possible Identity of the *mel(1)R1* and *yea 3-1* Loci.

Since *mel(1)R1*^{o}/*yea 3-1* females are semi-fertile, the fertility phenotype of these females is certainly not that of the *mel(1)R1*^{o} homozygous female. Falk (personal communication) has tested the fertility of *yea 3-1* homozygous females. Some females were sterile, and others produced reduced numbers of offspring relative to +/+ control

females. It has also been established that the sterility of yea 3-1 females shows no dominance: all heterozygous yea 3-1/Basc females give large numbers of male and female progeny. Thus the fertility phenotype of miel(1)Rl^{o}/yea 3-1 females appears to be similar to that of yea 3-1 homozygotes. This would indicate that miel(1)Rl and yea 3-1 may be the same locus and further testing is warranted.

Independent evidence for a nutritional response to yeast by mutations at the miel(1)Rl locus was mentioned earlier: yeast-fed homozygous miel(1)Rl^{o} females produced about twice as many post-blastoderm lethal embryos as did non-yeast-fed females.

III. THE PHENOMENON OF GENETIC RESCUE OF MATERNALLY INFLUENCED EMBRYONIC LETHALITY.

The phenomenon of genetic rescue is exhibited by alleles of a number of loci mainly on the X-chromosome (see Introduction). While the genetics of these mutations is clear (Lynch, 1919), their physiology is very much a matter for speculation at this time (Wright, 1973; Fausto-Sterling, 1971a; Shannon 1972a; Counce, 1956a).

A number of characteristics of mutants at these loci need to be considered in order to make hypotheses about the physiological role of the loci.

Several of these loci exhibit both pleiotropy of mutant effects and multiple allelism. In the cases of cin and dor the production of abnormal eye pigmentation and female sterility are clearly biochemical correlates of some primary genetic defect. However, in neither case has it been demonstrated that the pigment or any of its

precursors is directly responsible for the maternally influenced embryonic lethality. fu (Lynch, 1919; Counce, 1956b; Fausto-Sterling, 1971a,b) exhibits morphological pleiotropy - fusion of wing veins, and a high incidence of ovarian tumours, but no chemical correlates of fu mutants have been discovered. In rudimentary a similar situation held until it was discovered that this locus controls a step (or steps) in pyrimidine synthesis (Norby, 1973). That defects in pyrimidine synthesis lead directly to the infertility of rudimentary mothers, either directly through low pyrimidine pools, or through an insufficient supply of the requisite metabolic enzymes, in embryos is supported by several observations. Bahn (1970) has shown that fertility of r females can be enhanced by feeding them on pyrimidine enriched medium. Schneiderman (unpublished) has also demonstrated that genetically lethal embryos produced by the mating of r females and r males can be rescued by injecting them with either pyrimidines or cytoplasm from wild-type eggs.

Many of the loci that can mutate to give genetically rescuable miel effects, notably fu, dor, miel(1)R1, and especially r, have more than one mutant allele. In general, the severity of defects caused by a mutation at one of these loci can vary in expression from recessive lethality through limited female fertility. Perhaps the best example of this range of mutant effects is the dor locus (Bischoff and Lucchesi, 1971).

For each locus, all the pleiotropic effects of a mutant allele correlate in severity with the severity of the female sterility. This has been well demonstrated by Carlson (1971) for the r locus, and extended by Falk (1973). Falk rank-ordered a number of mutant alleles by the expression of their most sensitive phenotype, pyrimidine auxotrophy. The expression of the other phenotypes, wing morphology

and female sterility, correlated with the degree of auxotrophy.

Two of the mutants, r (Norby, 1970) and yea 1-1 (Falk, 1973) are nutritionally supplementable auxotrophs. Another auxotrophic mutation, yea 3-1, isolated by Falk, may be an allele of mie1(1)R1^{r} and mie1(1)R1^{o}. Nash, Falk, and Romans (unpublished) have found that the incidence of female sterile mutants among auxotrophs is too high to be due to chance.

All of the results on the loci are consistent with the hypothesis that these mutants are defective in macromolecules involved in the general metabolism of the fly rather than in specialized structural elements important in morphogenesis. The biochemistry of the r locus, and that known for dor and cin bear out this suggestion. The maternally influenced embryonic lethality could be associated with any of four possible defects in oogenesis: the oocyte does not receive sufficient amounts of 1) an essential nutrient 2) essential derivatives of such nutrients 3) the enzymes for nutrient production or 4) messenger RNAs for the production of these macromolecules. Genetic rescue can occur when the locus in question can be transcribed during embryogenesis, and does occur if the mutant cytoplasm is sufficiently normal to support development until the wild-type product is available.

In this view, mie1(1)R1^{r} and mie1(1)R1^{o} are simply two mutations of different severity at a locus whose function occurs in oogenesis and during embryogenesis, and which might be revealed to function at other times in development as well, through a study of other mutant alleles.

The lem locus in Bombyx mori has two mutant alleles which parallel the mie1(1)R1 locus very closely. This is also the only locus that shows a maternal effect for which the biochemistry has been well studied. lem appears to be the structural locus for pteridine reductase (Tsujita, 1961; see also Wright, 1970). lem/lem moths are viable and show low levels of the enzyme activity. Their yellow colour is due to the accumulation of sepiapterin. lem^1/lem^1 individuals are lethal as yellow second instar larvae and have no enzyme activity. The enzyme shows a maternal effect: eggs derived from the crosses of + females with lem males show an increase in enzyme activity about two days earlier in embryonic development than eggs derived from the reciprocal cross. These results have been interpreted as suggesting that the maternal and paternal genomes are activated at different times (Wright, 1970). An alternative interpretation is that substances in the maternal cytoplasm, either inactive pteridine reductase or its messenger RNA, are activated two days prior to embryonic gene product.

Genetically rescuable maternally influenced embryonic lethal mutants can be used to ask some fundamental questions about embryogenesis. One important question deals with the genetic regulation of development, specifically the effects of the cytoplasm on the genetic material. This question has been hinted at in the discussions of Shannon (1971b), and Fausto-Sterling (1971b). Garen and Gehring (1972) have shown that the injection of wild-type cytoplasm into lethal dor embryos postpones their death until just before hatching. Another important question has to do with possible separate control of maternally and paternally derived genes. It has been shown for lem in Bombyx

(Tsujita, 1961) that genes from the two sources do not begin to function at the same time.

Wright (1973) has suggested that selection for cold sensitive genetically rescuable miel mutants should delimit some ribosome defective mutants. On the basis of our knowledge of the source and function of ribosomes in Drosophila embryos, and that ribosomal mutations have been isolated as cold sensitive E. coli mutants (Nomura, cited in Wright, 1973) this is not improbable. Certainly, several mutations that are genetically rescuable maternally influenced embryonic lethals are also cold sensitive recessive lethal mutations.

As it now stands, those loci which yield genetically rescuable maternally influenced embryonic lethal mutations clearly are of great practical importance to the study of development. However their function as fundamental morphogenetic determinants is questionable, and it seems more likely that their role is in cellular metabolic processes which attain vital significance in the cataclysmic and environmentally isolated events of embryogenesis.

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APPENDIX

ISOLATION AND PRELIMINARY CHARACTERIZATION OF X-LINKED FEMALE STERILE MUTATIONS

The two mutants mei*l*(1)R1^{r} and mei*l*(1)R1^{o} which are the subject of this thesis were selected according to the procedure outlined in Figure 2. Approximately 780 X-chromosomes were tested and a total of twenty-nine mutations resulting in female sterility or semi-sterility were isolated. Those stocks in which no female produced any adult offspring are referred to as "female sterile", and the stocks in which some females were capable of producing a few offspring, "female semi-sterile". Some characteristics of these mutants will be described in this Appendix.

The phenotypes of the mutants are shown in Table A1. Fertility was scored in ten crosses of one homozygous females with three hemizygous males. Each vial was examined for 1) presence or absence of eggs, 2) pre- or post-blastoderm death of embryos (by the criterion that post-blastoderm lethal embryos turn brown, Würgler, 1968) and 3) semi-sterility. The ability of an X-chromosome bearing the wild-type allele of the mutation to genetically rescue a mutant was tested in a cross of three homozygous females with several Basc males.

All of the female sterile mutants except 126X1D, 88B2-3, and L109 were mapped using the y cv v f chromosome, as outlined in Figure 3. Since only twenty females recombinant between a given pair of markers were tested for sterility, accurate map positions of the mutants relative to the markers could not be calculated. However it was possible

Table A1. Recovered x-linked female sterile mutants.

Mutant Stock	Phenotype
L109/Basc	lays no eggs
M253/Basc	lays no eggs, ovaries appear to develop normally
G277/Basc	lays few eggs, preblastoderm lethal
L229/Basc	" " " "
83B2-1/Basc	" " " "
88B2-3/Basc	" " " "
L186/Basc	lays normal numbers of eggs, preblastoderm lethal
L193/Basc	" " "
L196/Basc	" " "
L211/Basc	" " "
M382/Basc	" " "
M400/Basc	" " "
125X2B/Basc	" " "
126X1A/Basc	" " "
126X1D/Basc	" " "
12N2X2D/Basc	" " "
M163/Basc/FM7b	late embryonic lethal
L207/Basc/FM7b	late embryonic lethal, genetically rescuable
M69/Basc	some females fertile
M214/Basc	" " "
M388/Basc	" " "
85B1-1/Basc	" " "

Table A1 continued.

Mutant Stock	Phenotype
87B1-2/Basc	some females fertile
162B2-3/Basc	" " "
166B2-2/Basc	most females lay very few eggs, some hatch
166X1C/Basc	some females fertile
164X2B/Basc	most females lay no eggs, a few females lay eggs, some of which survive to adulthood
82X1A/Basc	garnet mutant, some females fertile
M226/Basc	a few females produce a few offspring, semi-dominant

to determine whether each mapped relatively close to the proximal marker, half way between the markers, or close to the distal marker. The data are shown in Table A2 and the approximate locations of the mutants are summarized in the diagram of the X-chromosome shown in Figure A1.

All the mutant stocks classified as female sterile, and several of the semi-steriles were tested for alleism using the complementation test illustrated in Figure 4a. The crosses were made in bottles, ten males and ten females in each cross.

The results of these complementation tests are summarized in Table A3. Although the complete set of reciprocal crosses indicated by Figure 4a was carried out, I have shown only those results corresponding to Crosses 1 and 2 in Figure 4a. The complementation data not reported in the table were consistent with the reported results. The result of a test was similar independent of which mutant of a given pair was the heterozygous female parent in the P generation with only one exception, M226, which will be discussed later. Furthermore only those crosses involving mutants which mapped in the same map interval are shown, since there were no inconsistencies between the map position (Table A2, Figure A1) and the complementation data. Thus, for example, a mutant mapping between v and f complemented with all those tested that map in any other map interval. Complementation t4sts with known female sterile mutants were carried out in some cases where mapping studies justified such tests.

The following picture of the induced mutants arises.

Eighteen of the mutants, about 60% could be classified as female sterile and the remaining mutants are semi-sterile. Of the sterile mutants, only

Table A2. Map intervals of female sterile mutants.

\+ = fertile

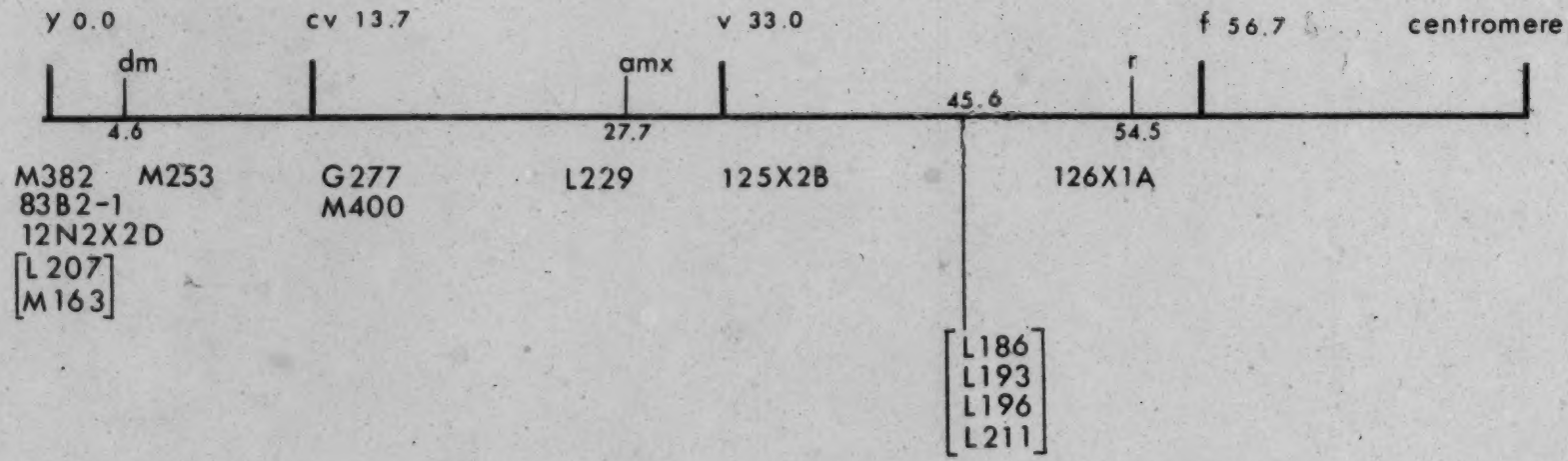
\- = sterile

? = can best be explained by a double crossover

Table A2 Map intervals of female sterile mutants

fertility of recombinant classes	L207	M163	M382	12N2X2D	83B2-1	M253	G277	M400	L229	126X1A	L186	L193	L196	L211	125X2B
	0-	0-	0-	0-	1-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-
y cv v f	10+	10+	10+	10+	8+	10+	10+	8+	8+	10+	10+	10+	10+	9+	10+
	10-	10-	10-	10-	10-	10-	10-	10-	10-	10-	10-	8-	10-	10-	10-
+ + + +	0+	0+	0+	0+	0+	0+	0+	0+	0+	0+	0+	0+	0+	0+	0+
	0-	0-	0-	0-	0-	0-	1-	0-	0-	8-	4-	3-	7-	3-	8-
y cv v +	10+	10+	9+	10+	10+	10+	8+	10+	9+	1+	6+	6+	3+	7+	1+
	10-	9-	7-	9-	10-	10-	8-	10-	9-	1-	3-	5-	2-	3-	1-
+ + + f	0+	0+	0+	0+	0+	0+	0+	0+	0+	9+	6+	4+	6+	7+	9+
	0-	0-	0-	0-	0-	0-	1-	1-	7-	10-	9-	10-	10-	10-	10-
y cv + +	10+	10+	10+	10+	10+	10+	9+	9+	3+	0+	?1+	0+	0+	0+	0+
	10-	3-	10-	10-	9-	9-	8-	7-	0-	0-	0-	0-	0-	0-	0-
+ + v f	0+	0+	0+	0+	0+	?1+	2+	2+	7+	10+	9+	10+	10+	10+	10+
	0-	0-	1-	0-	0-	3-	10-	9-	7-	9-	10-	10-	10-	10-	9-
y + + +	10+	10+	9+	10+	8+	7+	0+	0+	0+	?1+	0+	0+	0+	0+	?1+
	9-	3-	10-	8-	6-	4-	0-	0-	0-	0-	0-	0-	0-	0-	0-
+ cv v f	1+	2+	0+	2+	0+	6+	6+	9+	10+	8+	3+	9+	10+	10+	10+
map interval	y - cv close to y	=	=	=	=	y - cv middle interval	cv - v close to cv	=	cv - v close to v	v - f close to f	v middle interval f	=	=	=	v - f close to v

Figure A1. Approximate map positions of female sterile mutants compared to previously described female sterile mutants.



Allelism ( ) was determined from data in Table A3 a and c.

Table A3. Allelism among female sterile and semi-sterile mutants.

a Mutants mapping between y and cv (Table A2)

fs^1 parent \ fs^2 parent	M163	L207	M382	M253	12N2X2D	83B2-1	dm
M163	-	-	+		+	+	
L207	-	-	+	+	+		
M382	+	+	-	+	+	+	+
M253		+	+	-	+		
12N2X2D	+		+	+	-	+	
83B2-1	+		+		+	-	
dm							-

+ = fertility, - = sterility (non-complementation)

The table shows the results of Crosses 1 and 2 as outlined in Figure 4a.

Table A3 continued. Allelism among female sterile and semi-sterile mutants.

b. Mutants mapping between cv and v (Table A2)

fs^1 parent \ fs^2 parent	G277	M400	L229	amx
G277	-	+	-	+
M400	+	-		+
L229	+		-	
amx	+	+		-

Table A3 continued. Allelism among female sterile and semi-sterile mutants.

c. Mutants mapping between v and f (Table A2)

fs^1 parent \ fs^2 parent	125X2B	L996	126X1A	L186	L211	L193	r^9
125X2B	-		+	+	+	+	
L196		-	+	-	-	-	+
126X1A	+	+	-			+	
L186	+	-		-	-	-	+
L211	+	-		-	-	-	+
L193	+	-	+	-	-	-	+
r^9							-

Table A3 Continued. Allelism among female sterile and semi-sterile mutants.

d. Crosses of mapped mutants with unmapped mutants

fs^1 parent unmapped mutant	fs^2 parent, mapped mutant: M253	M382	G277	M400	12N2X2D	L186 alleles	126X1A	125X2B	126X1D
162B2-3			+			+			+
126X1D									-
M226		+	+	+	+	+	+	+	
M388	+				+	+		+	
L109				+			+		
85B1-1									

two mutants L109 and M253 produce homozygous females which fail to lay any eggs. Dissection of ovaries of homozygous M253 females shows that, superficially, at least, these ovaries develop normally, although eggs are never laid. The rest of the genitalia of these females were not examined, so it is not known if the failure to lay eggs is due to anatomical abnormalities. It is not known whether M253 and L109 are allelic.

Fourteen sterile mutants, comprising eleven probable loci (four are allelic to each other) produce homozygous females that lay eggs which die before blastoderm completion. The four mutants which are allelic are L186, L193, L196, and L211. Since these four are alleles, the data from the mapping experiments (Table A2) could be pooled and an approximate map location calculated, using the relationship given by Nash (1963). The recombinant classes are

v + +	22
v fs +	17
+ + f	23
+ fs f	13
Total	75

The standard map positions of v and f are those given in Lindsley and Grell (1968), and substitution into the equation of the values a = 40, b = 35, x = 23.7, Z = 33.) gives an approximate map position of 45.6. The 95% confidence limits, (see Nash, 1963) were calculated to be 43.0 and 48.2.

Since this locus maps in the same interval as rudimentary (54.5), a known locus affecting female fertility which also has a very high mutation rate, complementation tests were performed between L186, L193, L196, and L211 and r^9 (Table A3c). Females homozygous for r^9

are essentially sterile (one female of 14 tested in this laboratory gave one offspring, when mated to $\underline{r^9}$ males). All tested females heterozygous for one of L186, L193, L196, and L211 and $\underline{r^9}$ were fertile in crosses with either male, giving both male and female offspring. Although $\underline{r^9}$ is not an allele of r which gives non-complementation with all other alleles throughout the locus (Carlson, 1971), in view of the results and the map position of the locus described here, it is unlikely that the four mutations tested are alleles of rudimentary.

Among the true female sterile mutants, the remaining two, L207, and M163, proved to be lethal late in embryogenesis, after blastoderm formation. Although their phenotypes were different in that some L207/+ zygotes produced from the mating of homozygous L207 females and +/Y males survived and eclosed as adults, while the comparable M163 cross was completely sterile, these mutants proved to be allelic.

The pattern of fertility among the semi-sterile mutants was generally the same for all the mutants, in that about one half the females produced one to ten adult progeny each. However, three mutants revealed additional phenotypes:

1) Complementation tests involving M226 showed that M226 is somewhat dominant and also affects male fertility. The fertility of crosses was as follows;

fs/M226 x fs/Y > fs/M226 x M226/Y > M226/fs x fs/Y > M226/fs x M226/Y

"fs" in this case refers to all the other mutants tested with M226, and the convention followed is that the female fs^1/fs^2 obtained the fs^1 chromosome from her mother.

2) 82X1A homozygotes and hemizygotes have a purple-red eye colour which has been found to be garnet by mapping and by complementation. These flies are also smaller than heterozygotes. When this stock was last examined some homozygous females were fertile, although none were in a previous examination. Consequently the female sterility is hard to map.

3) Homozygous females and hemizygous males of the stock 164X2B emerge late and are few in number relative to the Basc heterozygotes and Basc males also present in the stock. When the homozygous mutant females were tested for fertility, few laid any eggs. Some embryos of those which were capable of laying a few eggs did develop further. Most of these progeny died as larvae or as pupae but a few adults eclosed, almost all females. The finding of this mutant as a female semi-sterile raises a question as to the relationship between recessive lethality in one generation, and infertility in the next. This mutant might perhaps be equally well described as a recessive semi-lethal, with the semi-sterility being simply a consequence of the tested females being already very sick.

A brief report by Mohler (1973) indicated that there are many more X-chromosome loci which yield female sterile mutations than have been identified in this mutant screen: 80 of about 200 female sterile mutations he isolated have been assigned to 34 loci. Mohler's screen and that of Bakken (1973) for female sterile mutations on the autosomes show that a great variety of defects, ranging from those affecting the early stages of oogenesis to those maternally affecting embryos late in their development, can be detected as female sterile mutations. Careful description and preservation of these mutant

stocks will provide the basic material for studies on the genetic regulation of embryonic development.